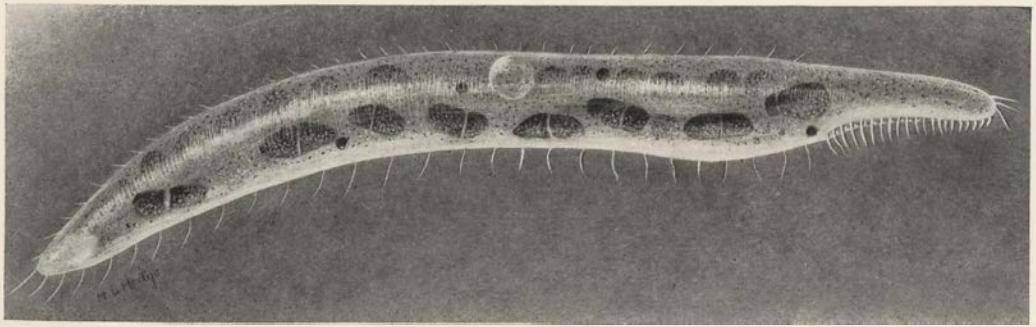

FIG. 1.—*Uroleptus mobilis.* (Drawn by Mabel L. Hedge.)

THE

BIOLOGY OF THE PROTOZOA

BY

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PREFACE.

Protozoölogy as a branch of the biological sciences, has meant little more than the application of biological or zoölogical methods to a definite but limited group of organisms, the Protozoa. The taxonomical, morphological and cytological aspects are well developed, and much progress has been made on the pathological side as well as on the side of general physiology, distribution and ecology. But there is no common aim, little common background and no common point of view through which these many aspects of Protozoa-study are woven together in any definite way to make a science of Protozoölogy. In this respect Protozoölogy differs from other branches of the Biological sciences. Bacteriology, for example, deals with miscellaneous minute organisms, but the science is well-knit through special technical methods and by serological aims. Genetics has for material the whole world of living things, but is more definite in its ends than almost any other branch of the biological sciences.

Knowledge concerning the Protozoa has developed upon one fundamental concept, viz.: that they are organisms of one cell. This, however, has not been a unifying conception; indeed, through sophistry, even this common ground is questioned by some. As a student of the Protozoa for many years, and as a teacher, it seems to me that what is most needed in protozoölogy at the present time is a common point of view from which we may compare and evaluate the vast annual output of observations and experiments. For such a common viewpoint it is necessary, however, to go beyond the conception of the cell to the underlying and more fundamental principles of biology.

In the present work I have brought together the conclusions founded on thirty years of research on the Protozoa and on an equal number of years of teaching protozoölogy at Columbia University and recently at the Marine Biological Laboratory at Woods Hole. I venture to hope that the presentation may be a step towards the unification of the various phases of Protozoa-study and a suggestion of a common point of view in protozoölogy.

The underlying biological principle in this presentation is the irritability of protoplasm, combined with protoplasmic organization. This organization is specific for each type of living things and is

present in fundamental form in spores, cysts and eggs. Each such organization under appropriate stimuli undergoes differentiation through which the derived or visible organization is developed from the fundamental organization. Through irritability of protoplasm and reactions to internal stimuli arising through metabolic activities as well as through reactions to external stimuli, the fundamental organization is progressively changed. Such changes lead to reproduction by division whereby the changed organization is restored to the fundamental type. Other changes are cumulative and lead to special modifications of organization which we recognize as meiotic, gametic and zygotic phenomena, with accompanying processes of reorganization and restoration to the fundamental organization. Reorganization thus may be accomplished by division alone (for example animal flagellates) by parthenogenesis (endomixis in ciliates), or by fertilization phenomena. Such changes are cyclical in character and differ from other changes in fundamental organization (variation) which may be induced by permanent change in external environment, or by changed stimuli resulting from modifications of the germ plasm.

This conception is fully developed in the following pages. The various types of visible organizations which form the basis of classification are described and keys are introduced to aid in the placing of the more common genera. The fundamental vital functions are treated as manifestations of vitality. Life and vitality are treated as independent concepts—life as organization, and vitality as the activity of the organization. The various phases of vitality—youth, maturity, and old age—are consequences of changed or changing organization through continued metabolism. Death is disintegration of the organization.

To many friends and colleagues who have helped in the preparation of this work I wish to express my grateful appreciation. The illustrations, testify to the artistic skill of many different assistants, among whom I am particularly indebted to Miss Mabel L. Hedge (frontispiece and others), Mr. B. Manson Valentine, Mrs. Martha Clark Bennett and Miss R. Bowling. To the publishers, finally, I want to express my thanks and appreciation for their patience and good nature in waiting for a work long overdue, and for their coöperation and interest in the making of the book.

GARY N. CALKINS.

COLUMBIA UNIVERSITY, 1926.

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CHAPTER I.

INTRODUCTION.

A PROTOZOÖN is a minute animal organism, usually consisting of a single cell, which reproduces its like by division, by budding, or by spore formation and whose protoplasm has passed, or will pass, through various phases of vitality collectively known as the life cycle.

The maze of microscopic life to which the scientific world was first introduced by Anton von Leeuwenhoek in 1675 included a heterogeneous collection of animals and plants. Crustacea, rotifers, minute worms, diatoms and desmids as well as the more minute Protozoa, were all grouped together during the eighteenth and nineteenth centuries, first under the nondescript term *animalculæ* and later under the more descriptive term *Infusionsthiere* of Ledenmüller (1763). The correct zoölogical position of the higher types of animals was recognized before the middle of the nineteenth century and the group of strictly unicellular forms was first definitely outlined by von Siebold in 1848 under the name Protozoa, a term substituted by Goldfuss (1820) for Oken's suggestive *Urthiere* (1805), while the old name Infusoria has been retained for one of the subdivisions of the group.

The haziness in classification of the older zoölogists has not entirely disappeared in the light of modern knowledge and we are confronted today by the difficulties of distinguishing between Bacteria, unicellular Algæ, and unicellular animals or Protozoa. It is no reflection on modern science that we are unable to clearly differentiate between these three groups. To accept the problem as insoluble at the present time is merely to admit and apply our conviction that evolution is now, and has been in the past, the primary biological principle underlying the diversities of forms and functions of living things. Few biologists today will refuse to accept the view that higher types of animals—Metazoa—have been derived from forms in the past which were more or less similar to present-day Protozoa; or the view that higher plants have been evolved from unicellular plants. The variations and adaptations

which have been the stepping stones in this evolution have been and are still in progress among all types of unicellular things, so that no artificial definition of Bacteria, of Protozoa, or of Algæ will accurately distinguish either of these groups from the others. Haeckel (1866) undertook to avoid the difficulty by combining all unicellular forms under the common name Protista, but this is, obviously, only another name for the aggregate and an artifice for concealing the real difficulties which we should like to overcome. Minchin (1912), on the ground of structural characters, would distinguish Protozoa from Bacteria by the assumption that the latter are not of "cellular grade" because of the absence in many Bacteria of a typical cell nucleus. Here again, however, the old difficulty shows its head for in this sense, many well-recognized Protozoa are not, while many Bacteria are, of cellular grade (see Dobell, 1911). The problem after all has mainly an academic interest, and the chief practical value to be gained by its solution would be to set the limits of a text-book or monograph. We may reasonably expect to find therefore, in any treatise on Protozoa, some types which with equal right should be included in works on lower plants or on Bacteria.

It is less difficult to distinguish between Metazoa and Protozoa; the occurrence of a gastrula stage in the development of a questionable form is sufficient to place it unmistakably with the higher animals. Protozoa, indeed, are often associated in cell aggregates called colonies, the individual cells being held in place by protoplasmic connections, by stalk attachments, or by fixation in a common gelatinous matrix. In many cases these colonial aggregates resemble tissues of metazoa in their structural appearance, but tissue cells are dependent upon other parts of the animal for fulfilment of their vital activities while every cell of a colonial protozoön may be self-sufficient and independent, and differentiation among them is limited, at most, to reproductive and somatic cells (*e. g., Volvox globator, Pleodorina illinoisensis,* and their close relatives).

While the single protozoön is to be compared structurally with a single isolated unit tissue cell of a metazoön as a bit of protoplasm differentiated into cell body, or cytoplasm, and nucleus, it is a very different unit physiologically. In its vital activities it should be compared, not with the unit tissue cell, but with the entire organism of which the tissue cell is a part. All animal organisms perform the same fundamental vital activities of nutrition, excretion, irritability with movement, and reproduction, which are fundamental attributes of living animal protoplasm. In the higher types of Metazoa these primary activities are performed by complex organ systems, nutrition for example, involving not only the digestive system but the muscular, nervous, circulatory and respiratory systems as well. Each organ has its particular part to play in the

economy of the whole and each cell is differentiated for the purpose of its specialized function. Tissue cells, therefore, are physiologically unbalanced cells since they are preëminently specialized for secretion, or contraction, or irritability, etc. Division of labor in a physiological sense here reaches its highest expression.

In the lower Metazoa the organ systems are less highly specialized; fewer organs are present to perform the same fundamental vital activities and the tissue cells have relatively more kinds of work to do for the organism as a whole. Thus the supporting and covering cells of a cœlenterate combine the functions of respiration, irritability, muscular contraction, excretion and circulation with the primary functions of an epithelium. Each of them is more nearly balanced physiologically than a single cell of the higher types, but it still needs the activities of other cells, and the organism is again the sum-total of all its cellular parts.

In the protozoön, finally, we find a cell which is physiologically balanced; it is still a cell and at the same time a complete organism performing all of the fundamental vital activities within the confines of that single cell. Whitman, in his essay on "The Inadequacy of the Cell Theory" (1893) clearly expressed the inconsistencies in the common use of the designation "cell" for this variety of structures, and later writers, notably Gurwitsch (1905) and Dobell (1911) have followed in a similar vein.

As organisms the Protozoa are more significant than as cells. In the same way that organisms of the metazoan grade are more and more highly specialized as we ascend the scale of animal forms, so in the Protozoa we find intracellular specializations which lead to structural complexities difficult to harmonize with the ordinary conceptions of a cell. In perhaps the majority of the Protozoa the fundamental vital activities are performed, as in the simpler Amœbæ or simple flagellates, by the protoplasm as a whole and without other visible specializations than nucleus and cell body. In other forms, however, intracellular differentiations lead to intracellular division of labor which in some types becomes as complicated as are many of the organisms belonging to the Metazoa. Thus *Diplodinium ecaudatum*, one of the Infusoria, according to Sharp (1914) has intracellular differentiations of extraordinary complexity (Fig. 2). Bars of denser chitinous substance form an internal skeleton; special retractile fibers draw in a protrusible proboscis; similar fibers closing a dorsal and a ventral operculum; other fibrils, functioning as do nerves of Metazoa, form a complicated coördinating system; cell mouth, cell anus, and a fixed contractile vesicle or excreting organ, are also present. All of these are differentiated parts of one cell for the performance of specific functions, and all perform their functions for the good of the one-celled organism which measures less than $\frac{1}{250}$ inch in length. Analogous, if not so com-

plete intracellular differentiations are present in the majority of Infusoria, while many of the flagellates, notably the Trichonymphidæ, have an almost equally elaborate make-up. In all such cases the single cell is a complicated mechanism and the coöperating parts have the same relation to the organism as a whole as do the organs

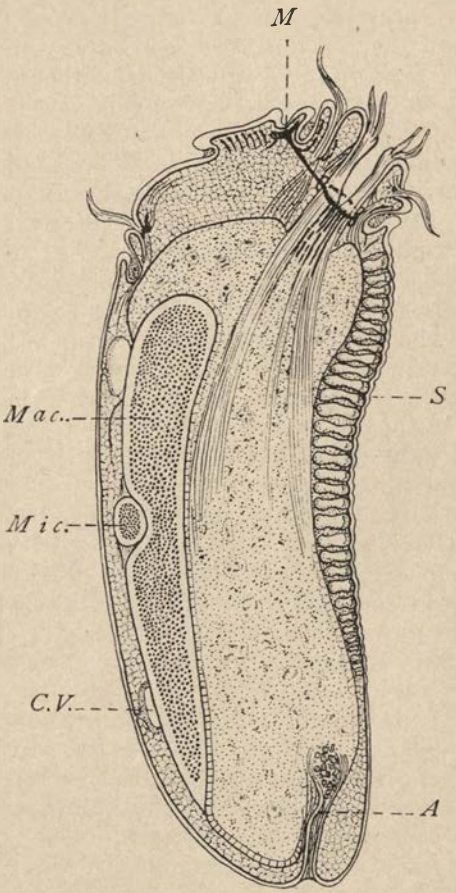

FIG. 2.—*Diplodinium ecaudatum*, a parasitic ciliate in cattle. *A*, anal canal and defecatory vacuole; *C. V.*, one of the two contractile vacuoles; *M*, motorium with fiber to circumpharyngeal ring; *Mac.*, macronucleus; *Mic.*, micronucleus; *S*, skeletal layer. (After Sharp.)

of a metazoön. Compared with an *Amœba proteus* or other simple rhizopod such complex organisms are highly specialized and show the extent to which intracellular differentiation may be carried. As Gurwitsch, Hartmann, Dobell, and others have pointed out, the application of the term cell which designates a structural unit with

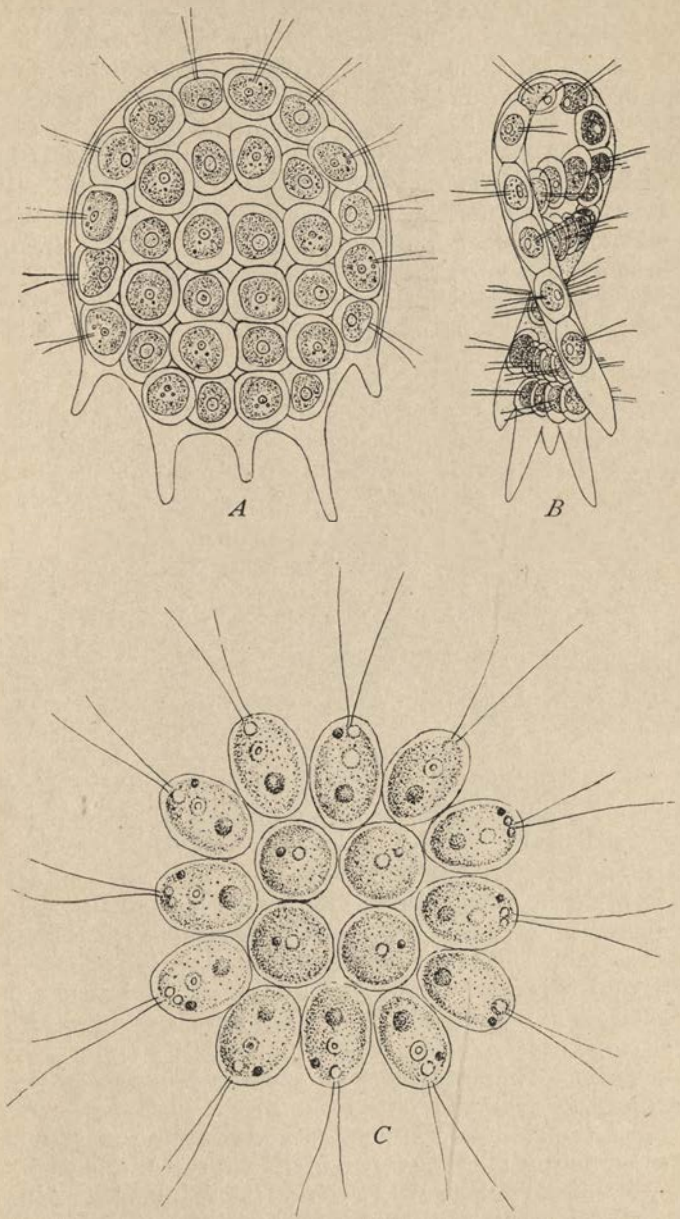

FIG. 3.—Types of Protozoan colonies. *A*, *B*, front and side views of *Platydorina caudata; C*, *Gonium pectorale*. (*A*, *B*., from Doflein after Kofoid.)

specific physiological activity in Metazoa seems to be inappropriate, and as Whitman argued, inadequate.

Cell aggregates or colonies are likewise highly variable in their functional specialization. While many of them consist of fortuitous groups of cells with dimensions varying with the number of individuals joined together (*e. g., Ophrydium versatile, Dinobryon sertularia*, etc.), others are definite in form, number of cells, and in arrangement (*e. g., Platydorina caudata*, Kof.). Here the colony as such has a distinct individuality and in some cases (*e. g., Gonium pectorale*) undergoes a definite developmental cycle (Fig. 3). Again some colonies composed of otherwise independent cells do not react as separate individuals but the colony reacts as a coördinated whole. Thus *Zoöthamnium arbuscula*, composed of many hundreds of indi-

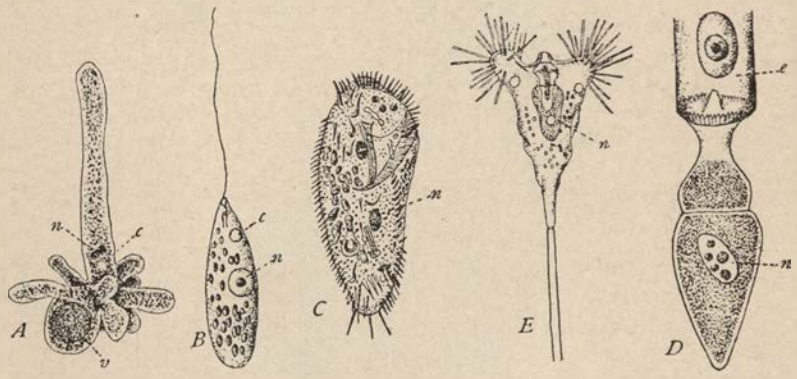

FIG. 4.—Types of Protozoa. *A*, *Amœba proteus*, a rhizopod; *B*, *Peranema trichophora*, a flagellate; *C*, *Stylonychia mytilis*, a ciliate; *D*, a polycystid gregarine; *E*, *Tokophrya quadripartita*, a suctorian. (*A*, after Calkins, *B*, *C*, *E*, after Bütschli; *D*, after Wasielewsky.)

vidual cells in a colony which may attain a diameter of 1 inch, reacts as a unit organism if any one of the component cells is irritated (Fig. 210). The entire aggregate contracts into a small ball, so minute that it is scarcely visible. The concerted action is due to the contraction of stalk myonemes which are continuous throughout the entire aggregate, like the cœnosarc of some hydroid colonies. For such colonies of protozoa, as for analogous colonies of hydroids, the expression "individual of a second order" has been applied.

Between the limits of the simplest and the most complex of unicellular organisms are the great majority of the (estimated) 15,000 or more known Protozoa. In each of the main subdivisions simplicity as well as extreme complexity of organization is represented, each subdivision including a series of representative forms ranging from one extreme to the other. Differentiations in the different

subdivisions do not follow the same lines of development, however, so that we are able to classify Protozoa according to a fairly natural system. These diverse lines of development make it difficult to treat this branch of the animal kingdom in any general way; the wide range in habitat from the purest waters of lake or sea to the foulest ditch, and adaptations to environments varying in character from a mountain stream to the semifluid substance of an epithelial, nerve, or muscle cell, has brought about manifold varieties of structure. To describe all of these modifications under a few headings, or to attempt to formulate general laws from the different and often highly complicated life histories, is out of the question. The general trends of differentiation, however, permit of grouping the different kinds of Protozoa in four types which were first outlined by the French microscopist Felix Dujardin in 1841. Three of these types—Sarcodina, Mastigophora, and Infusoria—are based upon the nature of the locomotor organs—pseudopodia, flagella, and cilia respectively—while a fourth type—Sporozoa—includes organisms which are invariably parasitic in mode of life and are essentially without motile organs (Fig. 4).

DISTRIBUTION OF PROTOZOA.

Protoplasm is an aggregate of fluid colloidal substances in which water plays a conspicuous part; exposed to the air it dries and desiccation is fatal to the majority of Protozoa, although it is possible that some forms, like certain rotifers, may reabsorb moisture and again become active. If the fluid protoplasm is surrounded by impervious membranes evaporation is prevented and within such capsules the protoplasm remains alive. This is the condition of encystment and many kinds of Protozoa, protected by their cyst membranes may live for long periods out of water (Fig. 5). Because of their light weight these cysts may be carried in the air and blown by the winds with dust, until surrounded again by water the organisms emerge from their cysts and are active once more for a few hours. Such encysted forms account in part for the surprising protozoan fauna in uncovered sterilized water in which food substances come from similarly protected germs of Bacteria and minute plant forms. Similar encysted forms may be present on the blades of dried grass, leaves, and other vegetation. In the infusions formed by soaking such dried vegetation in water various species of monads (*Monas, Oicomonas, Bodo*) and of ciliates (*Colpoda, Oxytricha, Stylonychia, Urostyla, Gastrostyla,* and *Vorticella*) and the rhizopod *Amœba* make their appearance in the order given (Woodruff, 1912). Puschkarew (1913) concluded that air-borne cysts play only a minor role, however, in the spread of Protozoa. It was found that on the average, there are

only $2\frac{1}{2}$ protozoön cysts per cubic millimeter of air and that these are limited to 13 species and represent the same types for the most part, as those listed by Woodruff. Protozoa are very apt to stick to solid substances when they encyst and are carried, in the dried state, with such substances, which accounts in part for the appearance of Protozoa in all kinds of infusions. Similar adhering cysts may be carried from place to place by birds and other flying creatures or by land animals thus helping to maintain a common type of proto-

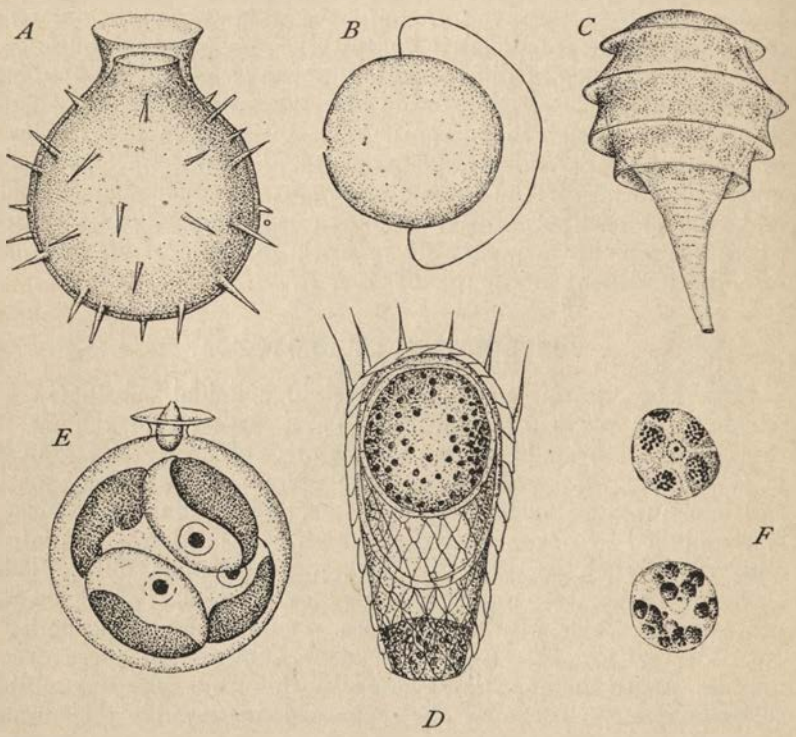

FIG. 5.—Types of Protozoan cysts. *A*, of *Ochromonas* sp; *B*, of *Hydrurus fœtidus; C*, of *Podophrya fixa; D*, of *Euglypha alveolata; E*, of *Chromulina pascheri; F*, of *Vahlkampfia limax.* (*A*, *B*, *E*, after Kühn; *C*, *D*, *F*., after Calkins.)

zoan fauna in pools and casual waters. Some forms to which Lauterborn (1901) has applied the term "sapropelic fauna," appear to be able to live without free oxygen. Thus *Frontonia leucas, Prorodon ovum, Spirostomum ambiguum, Pelomyxa palustris, P. binucleata,* etc., which usually live in relatively clear waters, may also live in the sulphurous medium of putrefying vegetable and animal matter, while certain species of ciliates of fantastic form, seem to require this peculiar habitat for their vital activities

(*Dactylochlamys pisciformis*, Lauterb., *Saprodinium dentatum*, Lauterb., *Discomorpha pectinata*, Levand., *Pelodinium reniforme*, Lauterb.). Doflein, following the suggestion made earlier by Bunge, believes that the anaërobic parasitic forms of the digestive tract may have had their initial start towards parasitism when living as such sapropelic forms.[1]

Protozoa are distributed over the entire world. Wherever there is moisture, there will these unicellular animals be found unless conditions of heat or of chemical composition are inimicable to life. Oceans and their tributaries, lakes, ponds, pools and ditches, mountain streams and wells contain them, their numerical abundance depending on the available food. They are present, not only in permanent waters but also in casual puddles of field and road, in droplets caught in the axils of leaves or in hollows of rocks, in rain water of roof or pail, and in damp moss. In many cases they are active for only an hour or more until their world dries up when they may again encyst, but some forms retain their activity in ordinary garden earth where they are supposed to play an important part in connection with Bacteria of the soil (Cutler and Crump, 1920, Goodey, 1916). The majority of such soil-dwelling forms belong to the Sarcodina and Mastigophora, Gruber's *Amœba terricola* being a typical case, while other genera and species are discovered from time to time (*Bodo*, *Prowazekia*, *Spironema*, *Oicomonas*, *Cercomonas*, *Nägleria punctata* and many others.

While excessive heat kills them, excessive cold does little harm beyond retarding vital activities and the melted ice of glaciers may teem with them, and some species are not harmed by exposure to liquid air.

They may live not only in the exposed waters of the earth's surface but also as parasites in the fluids of other living protoplasm or its products. They may be found in the warm blood of birds and mammals, or in the cold blood of fishes, amphibia and reptiles; in the digestive tract of every type of animal; in the saliva and urine of different types and in the living protoplasm itself of plants, other Protozoa, and of tissue cells. No type of animal life is free from the possibility of association with Protozoa either as commensals, or symbionts or parasites.

The common Protozoa of our own ponds and pools are exactly the same in genera and species as those found in similar places in

[1] The suggestive experiments and conclusions of Avery and Morgan (1924) give reason for the belief that the inability of some organisms to live in free-oxygen holding media is due to the absence in such forms of a peroxidase capable of breaking down hydrogen peroxide. The latter accumulates under ordinary aërobic conditions and is detrimental to forms which are unable to provide the peroxidase. The limitation of free oxygen may be the explanation of successful artificial cultivation of forms—for example *Spirostomum ambiguum*—which grow best under partly anaërobic conditions (see Bishop, 1923).

Europe, Asia, Siberia, Africa, South America and Australia; they are cosmopolitan, and the temptation to describe new species because they happen to have been found in some hitherto unexplored locality has no justification from the facts of geographical distribution. This is particularly applicable to the fresh water forms but does not apply equally to the deep sea types. The littoral fauna of salt water like the fresh water forms, appear to have a cosmopolitan distribution according to the observations of Gourret and Roesser (1886), of Levander and of Hamburger and Buddenbrock in Europe, and in North America where the brackish waters are particularly rich in number and variety of Protozoa. The pelagic and deep sea forms appear to be unequally distributed; some types are apparently limited to the Indian Ocean; others to the Atlantic, while many tropical genera and species, especially of Radiolaria and Foraminifera, are not found in the polar seas and *vice versa*. Some strictly pelagic forms on the other hand, notably *Noctiluca miliaris*, are found on or near the surface of sea water in all parts of the world.

Observations are sufficiently numerous to show that not only is there a certain climatic distribution of salt water forms, but a vertical distribution as well. Certain genera and species of Radiolaria and Foraminifera are present in the surface waters but are never found at the depth of from 600 to 3000 feet, while some families, notably the Challengeridæ and Tuscaroidæ, are present only in the extreme depths of the sea.

Many species are sufficiently adaptable to live either in fresh, brackish or salt water; indeed most of the common forms of rhizopods, flagellates and ciliates seem to be equally at home in either. Many types, however, sometimes entire groups of Protozoa, are not so ubiquitous; the sub-class Radiolaria for example, comprising more species than any other entire class of Protozoa, are exclusively marine, while another large sub-class of the Sarcodina, the Foraminifera, comprises only a few fresh water representative species. Many more types of Dinoflagellata are present in salt than in fresh water. Ciliates are poorly represented in the deep sea, although one family—Tintinnidæ—is wonderfully rich in salt water forms while fresh water forms are uncommon. Heliozoa, another subclass of the Sarcodina, on the other hand, are typically fresh water forms with relatively few salt water representatives. Many forms, especially the chlorophyll-bearing flagellates, are too sensitive to live vigorously in stagnant waters but thrive in the pure water of lakes and reservoirs, a predilection on their part which frequently leads to offensive odors and tastes in natural drinking waters (*Uroglenopsis americana, Synura uvella*).

The distribution of parasitic forms belonging to all groups of the Protozoa, obviously follows the distribution of their hosts and we

know too little on this subject to generalize; where animals are segregated the opportunities for parasitism are enhanced while some climatic conditions are more advantageous than others for the spreading of germs. Thus the blood-dwelling parasites are more common in the tropics than elsewhere, the biological conditions favorable to the intermediate transmitting hosts being largely responsible for their numbers and variety.

GENERAL ORGANIZATION OF THE PROTOZOAN BODY.

Although Protozoa belong unquestionably to the microscopic world their sizes vary within wide limits. Some are large enough to be picked up with forceps (*Porospora gigantea*, up to 16 mm.) and many of the larger ciliates are easily visible to the unaided eye (*Bursaria truncatella*, *Spirostomum ambiguum*) while many smaller types can be seen by the trained eye as mere white specks which, in some cases, may be identified by their characteristic movements (*e. g.*, *Paramecium*, *Frontonia*, *Dileptus*, *Amphileptus*, *Loxophyllum*, etc.). At the other extreme in size are types which are barely visible even with the most powerful lenses of the microscope. From 8 to 16 such forms have ample room for existence in a red blood corpuscle (*Babesia canis*), or 200 to 300 may live simultaneously in a single infected liver or spleen cell of man (*Leishmania donovani*). Between these two extremes of size lie the majority of Protozoa. Their measurements are usually expressed in terms of "microns" or thousandth parts of a millimeter which are represented by the symbol μ each micron being $\frac{1}{25000}$ of an inch. Thus *Leishmania donovani* measures from 2μ to 4μ, *Paramecium caudatum* upward of 200μ, *Bursaria truncatella*, 1500μ, etc.

The same species frequently shows remarkable variations in size due to environmental conditions or to different stages in the life history. Thus normal specimens of *Paramecium caudatum* may measure from 175μ to 250μ when fully grown and similar variations are characteristic of all species. Environmental conditions, especially food conditions, are frequently responsible for changes in size and character of a species, often rendering them difficult to recognize and affording tempting opportunities for swelling the list of synonyms by new names for the abnormal forms. Thus *Dileptus anser* when starved has a very different size and character from the normal form (Fig. 6). Again, different normal stages in the life history of a given species are not infrequently mistaken for different species, largely because of difference in size. Thus *Uroleptus mobilis* (see Fig. 1), in its adult vegetative condition, measures about 150μ, but immediately after conjugation not only is it reduced by one-third in size, but its internal structure is entirely different from that of the usual form, while during the period of old age it

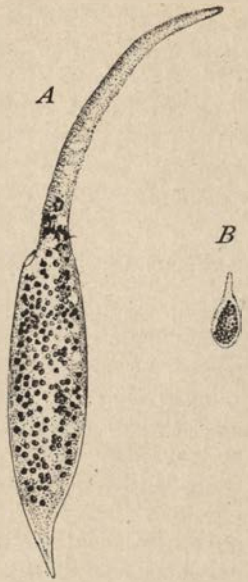

FIG. 6.—*Dileptus anser*, two sister cells. *A*, normal individual; *B*, individual starved for several days. (From Calkins.)

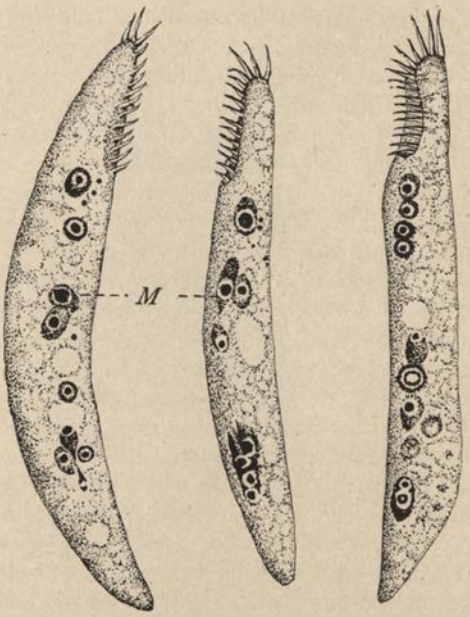

FIG. 7.—*Uroleptus mobilis* Engelm. Old age specimens showing degeneration of macronucleus *M* and loss of micronuclei. See frontispiece. (After Calkins.)

frequently measures less than 75μ (Fig. 7); and has a different appearance from the more youthful stages.

A. Form-relations of Protozoa.—The more important factors which determine form in Protozoa are: (1) The density or consistency of the protoplasm; (2) the presence of lifeless secretions and deposits in the form of membranes, shells and skeletons; (3) the mode of life; (4) the mode of reproduction; (5) inheritance.

(1) **Protoplasmic Consistency.**—All protoplasm contains the same fundamental chemical elements, C, H, N, O, and P which are necessary for the performance of vital activities. With these fundamental elements are associated mineral elements of one kind or another, Na, K, Ca, Mg, Fe, etc., usually as salts of different kinds and water. The physical properties vary with the composition in different cases and some types are more fluid, some more dense, than others. As a jelly-fish or medusa is obviously more fluid than the closely related hydroids or sea anemones, so it is with Protozoa. Some types are remarkably watery in their make-up while others are dense and stiff; a *Nuclearia delicatula* is much more fluid than *Amœba proteus*, and the latter more fluid than a *Pelomyxa palustris*.

These differences in consistency of the protoplasm have much to do with the form assumed by Protozoa, and more fluid forms, if not confined by resistant cell membranes, readily change in form according to environmental conditions, or by virtue of forces coming from metabolic activities within. *Amœba proteus* and other species of *Amœba* are amorphous and are constantly changing in shape, a characteristic phenomenon to which the term amœboid movement is applied, and the same protoplasm may be spherical in form, or flattened on the substratum, or extended in various ways. Many forms, under certain pressure conditions in the surrounding medium due to evaporation or reduced volume of water, will suddenly burst and disappear leaving no trace whatsoever of their previous presence. This phenomenon has been repeatedly mentioned by earlier observers in connection with types of Protozoa belonging to all classes, and the term *diffluence* was applied to it by Dujardin. In such cases the fluid protoplasm is usually confined by a resisting membrane or cortex which remains intact during the ordinary phases of activity but when the pressure from within becomes too great for the resistance of the membrane the latter collapses, the cell disappearing with all the characteristics of a miniature explosion.

Another evidence of the difference in density between different species of Protozoa is the reaction after cutting with a scalpel. Some species, for example *Paramecium caudatum*, are extremely difficult to cut successfully owing to the fluid character of the inner protoplasm which, as soon as the cortex is cut, flows out and disintegrates; in my experience not more than 20 per cent out of more

than 1000 operations on *Paramecium caudatum* have been successful, but the percentage is greatly increased by preliminary treatment with neutral red. Other forms of ciliates on the other hand may be cut in any plane, *Uronychia transfuga* and *Uroleptus mobilis* for example, reacting to such operations with all the physical properties of a piece of cheese.

The more fluid Protozoa, when the form is not maintained by resistant cortical differentiations react to physical properties of the surrounding medium. When forces on all sides are equal, as in

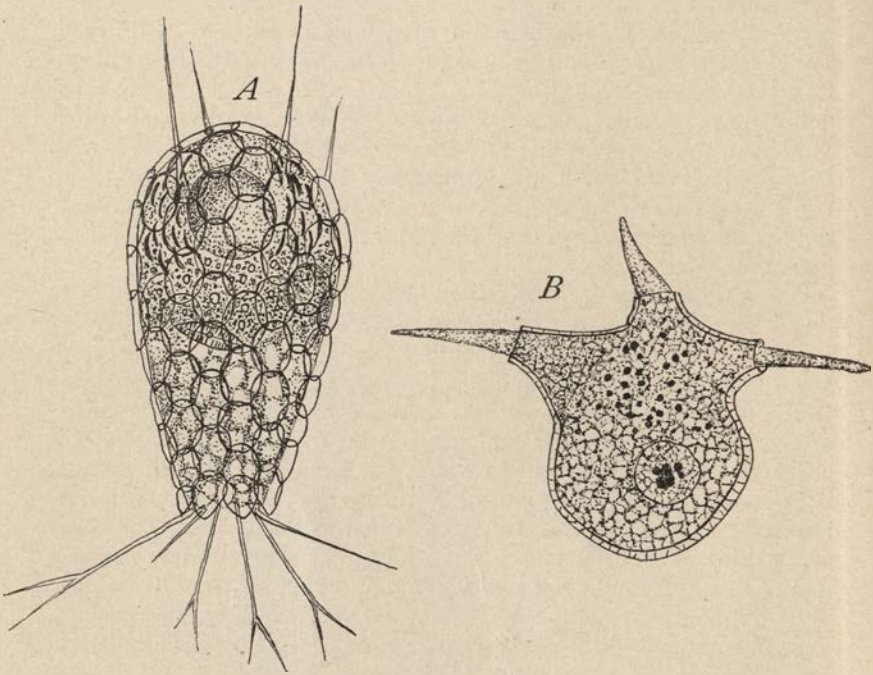

FIG. 8.—*Euglypha alveolata* (*A*), and *Cochliopodium* sp. (*B*). (After Calkins.)

suspended water-dwelling types like *Actinophyrs sol*, *Actinosphærium*, many Radiolaria, etc., the form is spherical, or spherical also in parasitic forms enclosed in the protoplasm of the host cell as is the case with the majority of Coccidia. In all types, under certain environmental conditions, or when continuously irritated, there is a tendency to become globular and this is the form assumed by the great majority of Protozoa when they encyst. The spherical, or homaxonic type, furthermore, is characteristic of the most generalized representatives of all classes of Protozoa.

(2) **Membranes, Shells and Skeletons as Form-determining Factors.**— While density or consistency of the protoplasm is thus one of the factors determining form in Protozoa, its effect in the majority of types is offset by the presence of definite membranes, shells, tests,

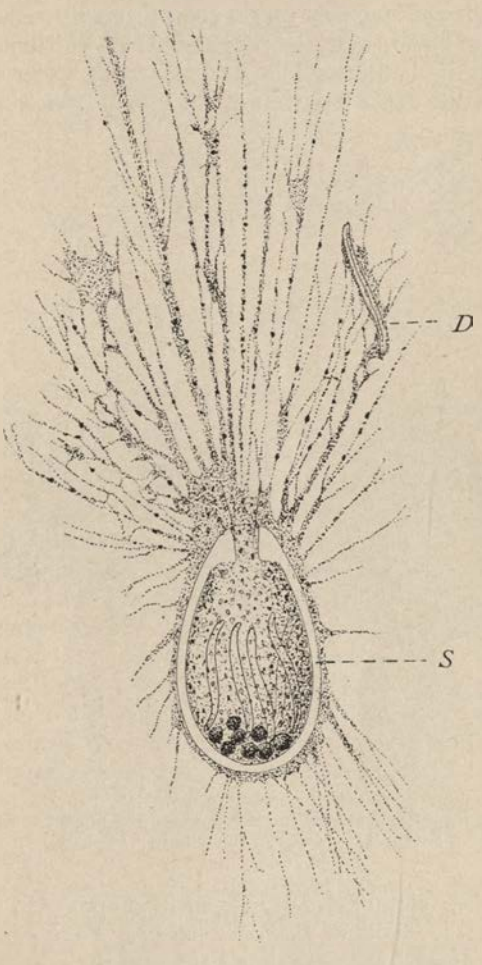

FIG. 9.—*Allogromia oviforme*, foraminiferon with chitinous monothalamous shell and reticulose pseudopodia. (*D*) a recently captured diatom; (*S*) chitinous shell. (From Calkins after M. Schultze.)

and skeletons; by specialized protoplasmic differentiations; or by foreign bodies. Thus the density of the sluggish *Pelomyxa palustris* is due to the enormous number of crystals of mud and sand, shells of diatoms and peculiar refractile bodies resembling glycogen in

make-up. Membranes of living substance, as in *Cochliopodium* (Fig. 8) and the majority of flagellates and ciliates; of lifeless chitin as in *Allogromia oviforme* (Fig. 9) or the lifeless materials secreted by the cell and deposited on it, are responsible for the forms assumed by many Protozoa. Even delicate types such as *Clathrulina elegans* and the majority of Heliozoa retain their forms by virtue of the protecting shells of lifeless materials deposited on a chitinous membrane. The protoplasmic bodies of many of the fresh water shelled rhizopods are relatively dense like that of the naked *Amœba verrucosa* and

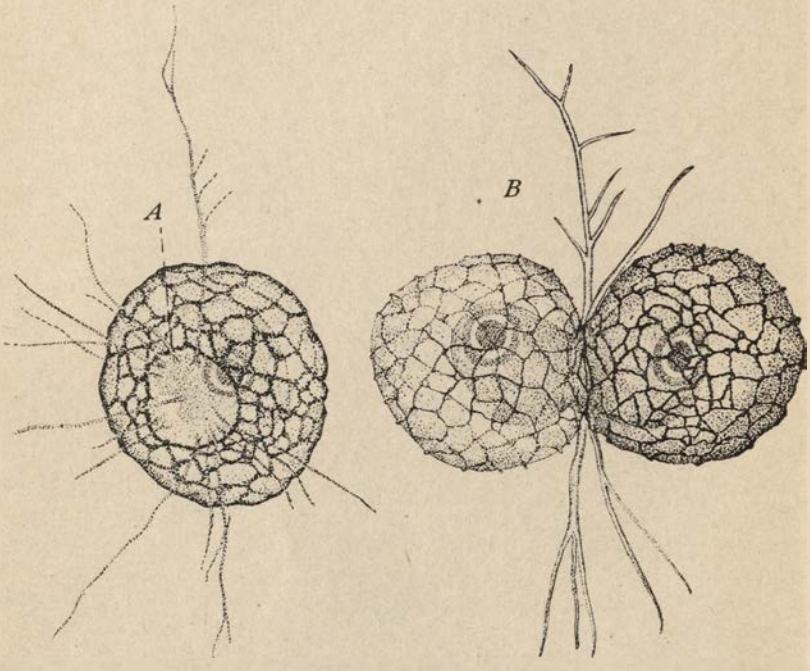

FIG. 10.—*Pseudodifflugia* sp. circular mouth opening and mosaic shell (*A*). *B*, division stage. (Original.)

are more or less globular or pyriform in shape. On such a protoplasmic basis the shells of *Difflugia* species, *Euglypha, Cyphoderia, Centropyxis, Arcella,* etc., are deposited and these, once formed, are never changed (Fig. 10). Only rarely are these shelled rhizopods flattened or discoid as in *Hyalodiscus.*

The typical form in many shell-bearing or skeleton forming rhizopods may be due in its last analysis to the finer structure of the protoplasmic body in which the skeleton or shell parts are deposited. Dreyer (1892) has given much evidence to show that the form and size of the elements making up the skeletal or shell parts depend

upon the alveolar make-up of the protoplasm, the interalveolar deposits of silica, etc., taking the form of spicules as in Heliozoa and many Radiolaria, of bars, hexagons, rings, fenestrated capsules, etc. (Fig. 11).

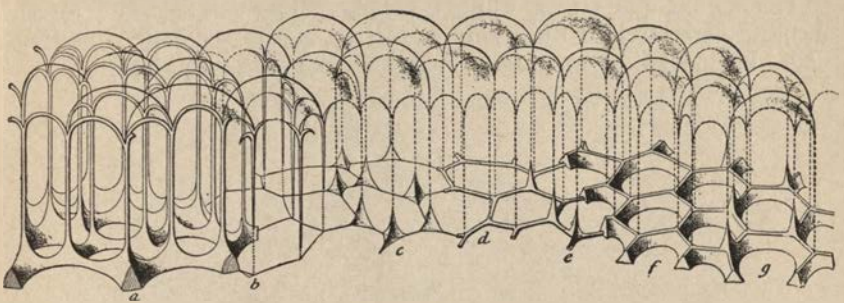

FIG. 11.—Schematic figure illustrating the modifications of skeletons according to mechanical principles of deposition. (After Dreyer.)

(3) **Mode of Life.**— A third factor determining form is the mode of life. As we have seen floating forms are usually homaxonic or spherical; freely moving types on the other hand are usually monaxonic. The type form of a freely moving flagellate or holotrichous ciliate is ellipsoidal, the cell being drawn out with its main axis extending in the direction of movement. Attached forms are usually polyaxonic or radially symmetrical, the variations in form depending upon the nature of the attaching portion. Some for example are attached by the protoplasm of the posterior end of a cylindrical body (*e. g., Cothurnia, Vaginicolla,* etc.); others by the more or less stalk-like attenuated end of the body (*e. g., Scyphidia, Podophrya,* etc.); and others by chitinous stalks of variable length (*Vorticella* species) which may be more or less branched (*Dinobryon* species, *Epistylis, Carchesium, Zoöthamnium,* etc.). In the same individual the form may change with change in mode of life, well illustrated by *Dimorpha mutans* (Fig. 12), by *Nägleria gruberi* or *Trimastigamœba.*

Methods of food-getting and the nature of the food are also potent factors in determining form. Many of the diatom- and desmid-eating ciliates, whose food lies on the bottom, are characteristically flattened forms with the mouth on the under, or physiological ventral, surface (holotrichous ciliates belonging to the genera *Chilodon, Orthodon, Opisthodon, Chlamydodon, Loxophyllum,* etc., and the majority of the hypotrichous ciliates). Special food-getting, or current-directing, organs frequently modify the form as in the collared flagellates (Choanoflagellates) and in types like *Folliculina ampulla, Bursaria truncatella,* cephalont gregarines, *Pleuronema,* etc. Shifting of the position of the mouth in response to different

food requirements has undoubtedly been the cause of some form changes as Bütschli has shown. Thus the proboscis-bearing species and the asymmetrical *Chilodon* types may owe their characteristic forms to such a shifting of the oral region (Fig. 13).

The monaxonic types, while typically ellipsoidal in form, are usually characterized by a spiral twisting of the cell body, especially in the rapidly moving forms. In some cases, notably in the

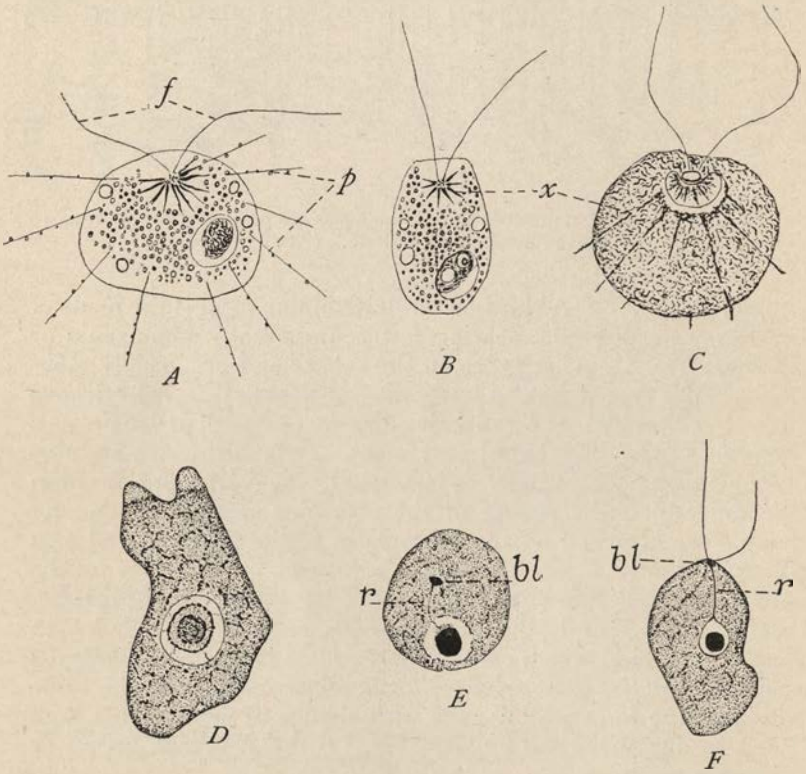

FIG. 12.—Diphasic rhizopods. *A*, *B*, *C*, heliozoa-like and flagellated stages of *Dimorpha mutans*. (After Blochman.) *D*, *E*, *F*, *Nägleria gruberi*, amœboid and flagellated stages; *E*, origin of blepharoplast (*bl*) from endosome; *r*, rhizoplast. (After C. W. Wilson.)

flagellates *Phacus longicauda, Phacus pyrum, Heteronema* sp., etc., and in the ciliates *Ægyria, Paramecium, Metopus sigmoides*, etc., the spiral twist is highly characteristic (Fig. 14).

Bilateral symmetry is of rare occurrence among Protozoa; indeed there seems to be only one really significant case, that of *Giardia* (Fig. 15). Here the two nuclei, the motor complex, and the eight flagella are arranged in the neatest bilateral manner. In some

colony forms, for example *Platydorina caudata,* thirty-two cells are aggregated in typical bilateral symmetry (Fig. 3, *A*). One possible mode of origin of such bilaterally symmetrical types is indicated by *Uroleptus mobilis* (Fig. 16). Here two individuals after conjugation, fused to form a single double individual which persisted through 367 generations (see also Fig. 194, p. 466).

(4) **Mode of Reproduction and Form.**— In this connection we have only to do with the multinucleated and with the colonial forms of Protozoa, for in ordinary division the daughter cells separate com-

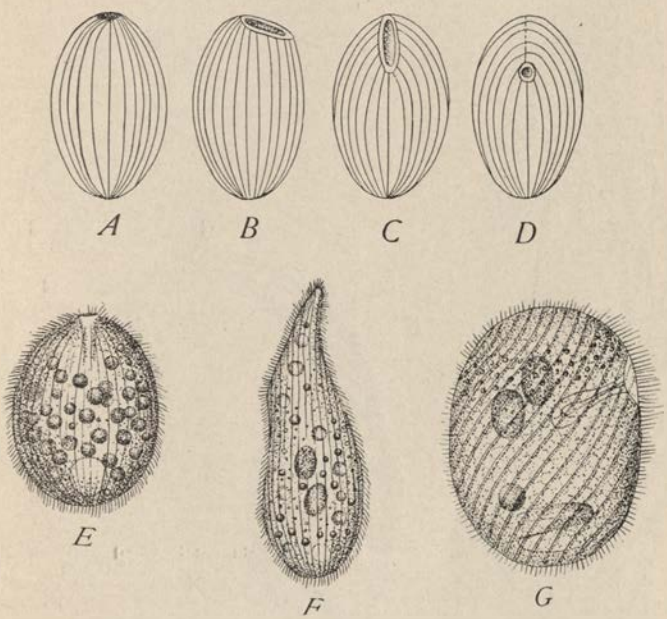

FIG. 13.—Diagrams illustrating shifting of the mouth in ciliates from terminal to lateral or ventral surface (*A, B, C, D*). *E, Prorodon griseus* corresponds with *A; F, Amphileptus claparedi,* corresponds with *B* or *C;* and *G, Nassula microstoma,* corresponds with *D.* (*E* and *F,* after Bütschli; *G,* after Calkins.)

pletely and reproduction has no effect on the form assumed. Thus the foraminiferon *Allogromia oviforme* gives rise by what is termed budding division to a free daughter cell which builds an independent test for itself while the other cell remains in the old test. In other forms of Foraminifera, however, the bud or protoplasm does not become separated from the parent bulk of the cell but takes a position in relation to the other portion which possibly depends upon the physical conditions of the protoplasm. New shells are deposited about the buds and a double chambered individual results (Fig. 17). Repetition of the process gives rise to distinct types of

polythalamous or many-chambered Foraminifera depending upon the position assumed by the bud (Nodosarine, Frondicularian, Rotaline types, etc.).

In colonial types the form of the aggregate is determined by the manner in which the individuals are held together after division. The different types are described as spheroid, catenoid, arboroid and gregaloid colonies. In the majority of spheroid colonies, the associated cells are held together by a gelatinous matrix secreted

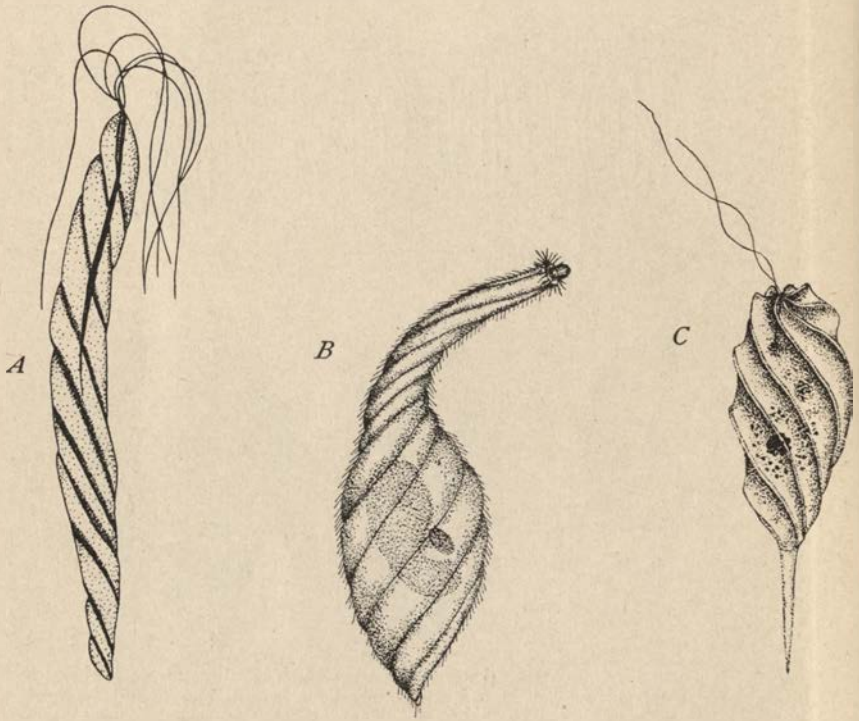

FIG. 14.—Types of spirally wound Protozoa. *A*, *Streblomastix strix*. (After Kofoid and Swezy.) *B*, *Lacrymaria* sp. (original); *C*, *Heteronema* sp. (Original.)

by the individual cells. The typical form of such colonies is spherical as in the various species of the genera *Volvox*, *Uroglena* (Fig. 18), *Pleodorina* and *Synura* among the flagellates, or *Ophrydium versatile* among the ciliates. Such spheroidal colonies, however, are not necessarily globular but may be flat plates of associated individuals as in *Gonium pectorale*, or *Platydorina caudata* (Fig. 3). In catenoid colonies the individuals are attached end to end as in some species of gregarines, or in the dinoflagellate *Ceratium*, or side by side as in the flagellates *Chlorodesmus* and *Chytridiastrum*, or in the ciliates

Haptophrya gigantea and *Polyspira delagei*. In arboroid colonies the individuals are attached directly end to end as in *Chlorodendron subsalsum* or by longer or shorter stalks in a branching, often bush-like colony [*Hyalobryon deformans* (Fig. 19), *Dinobryon sertularia* (Fig. 126, p. 529), *Epistylis umbellaria* (Fig. 210, p. 502),

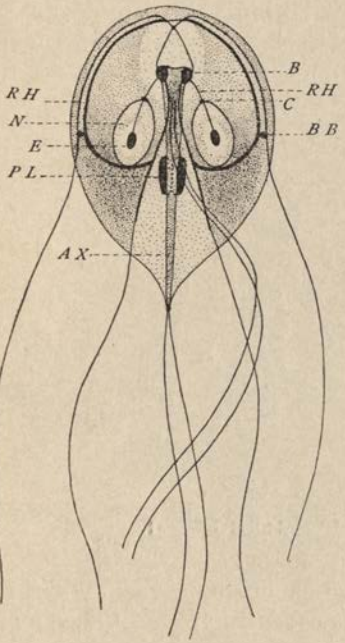

Fig. 15

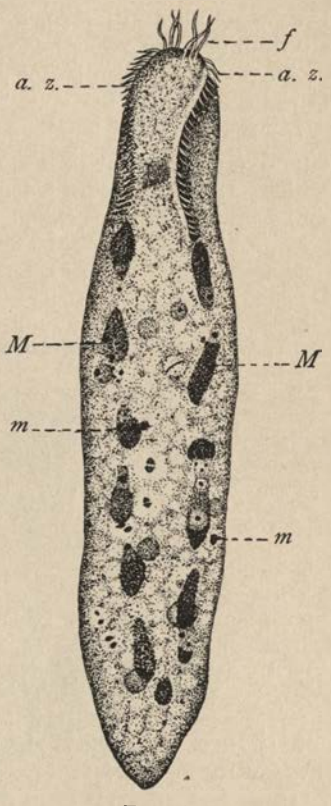

Fig. 16

Fig. 15.—A bilaterally symmetrical flagellate, *Giardia muris* Grassi. *AX*, axostyle; *B*, blepharoplast; *BB*, basal body; *C*, centriole; *E*, endosome; *N*, nucleus; *PL*, parabasal body; *RH*, rhizoplast. (After Kofoid and Swezy.)

Fig. 16.—A bilaterally symmetrical ciliate from *Uroleptus mobilis*. A double individual formed by fusion of two individuals after conjugating. With two mouths and adoral zones (*a. z.*); two sets of cirri (*f*); and two sets of macronuclei (*M*) and micronuclei (*m*). For structure of single individual see Frontispiece. (Original.)

Carchesium polypinum, *Zoöthamnium arbuscula*, etc.]. In the majority of these arboroid colonies each individual is borne on its own stem which branches from a common stalk. In some cases, however, especially amongst the flagellates, each stalk bears a cluster of individuals as in *Cladomonas fruticulosa*, *Anthophysa vegetans* or

Phalansterium digitatum (Fig. 20). In *Rhipidodendron splendidum* the gelatinous branches, colored brown or red by oxide of iron, are arranged in parallel rows, spreading out fan-like as they increase with division of the cells, the aggregate forming an organ-pipe-like arboroid colony. Gregaloid colonies, finally are fortuitous aggregates of previously independent individuals found mainly amongst the rhizopods and Heliozoa, or in parasitic flagellates under adverse environmental conditions (Spirochætes, Trypanosomes). The origin of gregaloid colonies is not connected in any way with the manner of reproduction.

(5) **Inheritance.**— The combination of all of the above factors effective throughout past ages, has resulted in fixed, complex forms which, as in Metazoa, are today associated with the germinal make-up of the protoplasm or genotype, and transmitted by

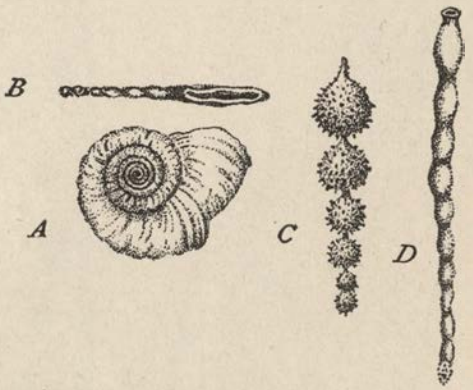

Fig. 17.—Types of shells of Foraminifera. *A*, *B*, side and ventral aspects of *Cornuspira* sp.; *C*, and *D*, types of *Nodosaria*. (After Carpenter.)

inheritance. Fantastic types such as *Discomorpha pectinata, Entodinium caudatum,* or *Phryocystis caudatus* are not uncommon.

In its last analysis form depends upon the chemical and physical make-up of the protoplasm and its polarity which signifies a specific protoplasmic organization and interaction of different protoplasmic substances. A minute fragment of *Uroleptus mobilis* is difficult to distinguish from a similar fragment of *Dileptus gigas,* yet the former develops into a perfect *Uroleptus* the latter into *Dileptus.* The encysted forms of many types are impossible to identify until the cysts are opened and vital processes begin again. These facts indicate that the finer or ultimate composition of protoplasm is different in different forms and specific for each species, and justify the view that there are as many kinds of protoplasm as there are species of Protozoa, Metazoa or living things generally. Considerations of this nature inevitably lead us into the lines of thought

followed by Whitman, Gurwitsch, Dobell, and many others and to question again the adequacy of the cell theory in its application to Protozoa.

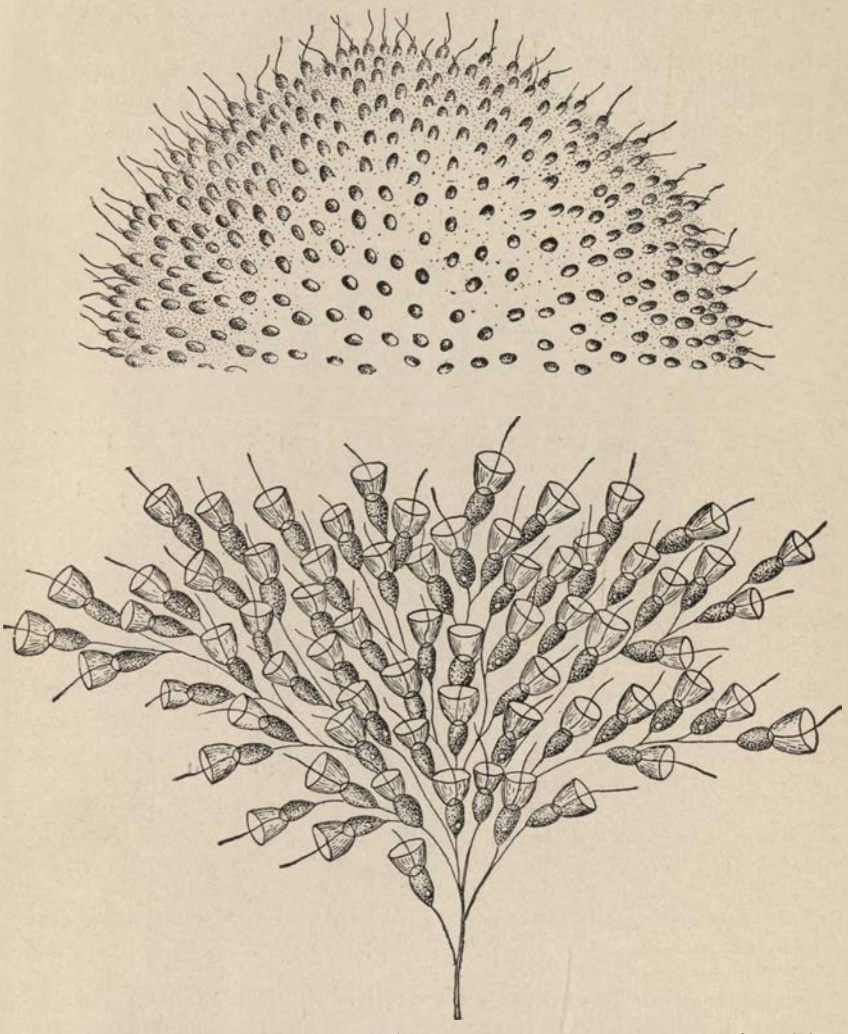

FIG. 18.—Types of flagellate colonies. *Uroglenopsis americana*, a spheroidal colony (above) and *Codosiga cymosa* Kent, an arboroid colony (below). (Former after Calkins; latter after Kent.)

B. Protoplasmic Structure.—The specificity of protoplasm is not at all indicated by its appearance although obvious differences in many cases may be seen even with low powers of the microscope. In a living form what we actually see under the microscope in most cases

with an accumulation of carmine granules where three planes of contiguous bubbles come together, while the spaces within the meshes would be filled with air. The apparent network, however, is merely the optical section of continuous walls of bubbles enclosed on all sides by the water and soap. The physical structure of the protoplasm of a few Protozoa, called spumoid structure by Rhumbler may be accurately compared with such an emulsion of soap and water. An analogous network, usually of exquisite fineness, represents the more solid substance of protoplasm; the apparent fibers forming the meshwork in some cases at least are the optical sections of continuous walls, which, like the soap bubbles, enclose materials of lesser density. Bütschli, who with Rhumbler, has studied the finer structure of protoplasm of lower plants and animals as well as that of higher forms, was the first to compare such structures with the alveolar structure of emulsions like soap and water, oils and water, etc. The granules of protoplasm, corresponding in position with the carmine of the soap suds, lie in the substance of the denser network of interalveolar material to which Doflein applies the term stereoplasm. The alveolar substance, called rheoplasm by Doflein, corresponds in position with the air of the soap bubbles.

All who have investigated protoplasm agree that it is not a homogeneous substance but a mixture of colloidal substances in the physical state described by Ostwald as an emulsoid in which the interalveolar materials act in the manner of a dispersing agent while the more fluid intra-alveolar substances are dispersed, but all are subject to reversal of phase.

While the alveolar structure of protoplasm is convincingly demonstrated by a number of typical forms of living Protozoa, this structure is difficult to make out in other types. Thus in the endoplasm of flagellates like *Chilomonas*, or the endoplasm of *Actinophrys sol*, or *Actinosphærium eichhornii*, the alveoli are easily discernible, but in *Paramecium caudatum*, in many gregarines, and in many types of flagellates and ciliates, the alveoli, if present, are too fine to be seen with the usual powers of the microscope. Vonwiller (1918) can find no evidence for upholding the alveolar theory of protoplasmic structure in general.

Certainly in many cases the protoplasm appears to be almost homogeneous in structure, the granules alone being evidence of structural configuration. Such forms are illustrations of the granula theory of Altmann, who held that protoplasm is made up of a congeries of such granules or microsomes each of which is termed a bioblast, each bioblast being regarded as a single unit performing all of the functions of living matter including growth and reproduction. Here, however, theoretical considerations have been superimposed on the obvious structures and the physical appearances

become clouded in a mist of speculation. Other theories, such as the reticular and fibrillar theories, associated with the names of Heitzmann, Schäfer, Flemming, etc., are based upon the actual pictures of different types of protoplasm.

The larger vacuoles in different types of Protozoa to which the names cavulæ and contractile vacuoles are given, are interpreted according to the alveolar theory as due to the flowing together and fusion of adjacent alveoli. This is certainly the case in the formation of a contractile vacuole of *Amœba proteus* where the beginnings of a vacuole may be watched under the microscope and the coalescence of minute vesicles noted. In a similar way the relatively hugh cavulæ or pseudo-alveolæ characteristic of *Actinosphærium eichhornii* and of Radiolaria may be accounted for.

Physically, protoplasm is to be compared with an emulsion of colloidal substances which, as Lord Rayleigh and others have pointed out, can as a polyphasic system, retain the emulsoid condition only as long as the limiting membranes between dispersed and dispersing media are intact. In the activities of a living, moving cell, there must be a continual disturbance of this physical equilibrium and a constantly changing configuration of the protoplasm due to the manifold chemical actions which are characteristic of living matter.

Chemically, protoplasm is not a substance but a harmoniously working aggregate of different interacting substances which have been identified in general as nucleins, nucleo-albumins, nucleo-proteins, carbohydrates, fats, salts, and the almost endless variety of derivatives from these and from their combinations. With the exception of the Mycetozoa which have been used extensively for the purpose of protoplasmic analysis, protozoan protoplasm owing to the minute size of the individuals, has been very little studied in connection with the chemistry of protoplasm, and our present knowledge concerning it is based mainly on morphological considerations together with the results of chemical analysis of protoplasm in higher types of animals and plants.

The granules which invariably appear in protoplasm, and which are probably intimately connected with the varied activities going on during life are different in their chemical make-up although, morphologically, they appear much the same. This is shown by their reactions to micro-chemical tests of different kinds and it is not unreasonable to infer that the specificity of protoplasm in different species of Protozoa is due in large part to the chemical and physical composition of these granules and interactions going on amongst them.

As Mathews points out, the essential differences in chemical actions in protoplasm and in physical nature are: (1) The orderliness with which they are carried on; (2) the speed of the reactions.

A starving *Dileptus anser* will slowly decrease in size although its form remains about the same. This is due to disintegration through continued oxidation and other catalytic processes which lead to the exhaustion of protoplasmic constituents unless new food is added. If the process is continued the organism will ultimately die in from one to three weeks. If a *Dileptus* is accidentally crushed its protoplasm will completely disintegrate within a few seconds. The process of disintegration in the first case is orderly, in the latter completely disorganized. Other normal vital activities are equally orderly; the orderliness dependent possibly on the regulation of permeability by the colloidal membranes, the alveolar membranes, nuclear membrane and investing membrane of the cell; and regulation of permeability in turn is dependent upon the chemical make-up of the constituent parts, and the salts or electrolytes and the continued activity between them (Cf. Clowes, Overton, Mathews).

The speed of specific chemical actions is a characteristic vital phenomenon due to the participation of subtle and elusive, but specific, catalytic agents, the enzymes.

This aggregate of colloidal substances forming polyphasic physical systems in protoplasm is the seat of the multitude of activities characteristic of life. Huxley's definition of protoplasm as the physical Basis of Life does not carry us very far in the analysis of living matter. Indeed it may well be that the physical basis of protoplasm is itself life (see Chapter IV) and that protoplasm in the words of du Bois Reymond, is the agent of vital manifestations. In a moving protozoön there is a constant interaction of the various substances making up its protoplasm—oxidation, enzyme formation and action, amidization and deamidization, disintegration and regeneration, protein break-down and protein reconstruction, all taking place simultaneously or *seriatim*. Substances in this whirlpool of action may be regarded as living so long as they are, or may be, drawn into the vortex of protoplasmic activities. The results of these multitudinous activities contribute to the well-being of one organism. In another moving protozoön a similar bewildering complex of activities likewise results in the well-being, in this case of a distinctly different type of protozoön. The first protozoön, let it be a *Didinium nasutum*, captures and swallows the second, say a *Paramecium caudatum*. It is well known that a fragment of a protozoön will regenerate into a perfect organism of its type and we might well be perplexed by the problem why is it that the *Paramecium* protoplasm in *Didinium* does not manifest itself as *Paramecium* and not as *Didinium*. The answer to this apparently simple problem is possibly a matter of organization or the manner in which the fundamental substances making up the protoplasm in the two organisms are put together and interact. The architecktonic of Driesch, or protoplasmic architecture is specific for each

type of organism and the form and structures of the organism are expressions of this architecture which is as perfect in a spherical fragment of a *Stentor* or of a *Dileptus* as it is in the fully developed *Stentor* or *Dileptus*. When this organization disintegrates, life and the possibility of controlled reactions are lost and the erstwhile living protoplasm becomes dead matter. This happens when *Paramecium* is paralyzed by the seizing organ of *Didinium* (see Fig. 89, p. 180). The vital activities of *Paramecium* are suddenly stopped, and disintegration of the protoplasmic organization of *Paramecium* continues with the process of digestion in *Didinium*. Then the inert proteins, probably as amino-acids, are reintegrated in the *Didinium* protoplasm and what was living substance in *Paramecium* now enters again, through a form of transmigration, into the vortex of vital activities of quite another type of organism.

Consideration of these and of similar activities in living protoplasm lead to questions regarding the nature of life and the nature of vitality. Should we use the two terms life and vitality as synonyms? It seems that there is something to be gained by distinguishing between them. We are very apt to speak of life as activity, or to say that life is a series of reactions, integrations and disintegrations. These may be manifestations of life but they are incomplete manifestations and do not tell the whole story. An encysted protozoön, a spore, a seed, a resting egg, or a dried rotifer, show no evidence of activity, yet each has life and in a proper environment would manifest activity. An emulsion of oil, salts and water, manifests activity strikingly similar to the movements of an *Amœba* yet such an emulsion has no life. The encysted protozoön or the dried rotifer has protoplasmic organization which the oil emulsion has not, and with absorption of oxygen and water becomes animated. Life thus is incontestably bound up with organization of protoplasm; perhaps life is best described as organization, thus giving it a static rather than a dynamic significance. Whatever name we give it, however, brings us no nearer to a conception of what it actually is, for life cannot be measured and endures until its organization is disintegrated. With vitality the case is different; here we have to do with protoplasm in motion and the activities can be measured from beginning to end of a life cycle. While life has evidently been continuous from the first protoplasmic organization, vitality has been intermittent or discontinuous. Life may exist without vitality and has always the potential possibility of vitality, but vitality is impossible without organization, *i. e.*, without life. I would define vitality, therefore, not as the same thing as life, but as the sum total of actions, reactions and interactions between and amongst the substances making up the organization of protoplasm and between these and the environment. It is in this sense that the term vitality will be used in the following pages (see Chapter X).

In a moving protozoön substances of different kinds are constantly involved and take part in the vortex of reactions. Many of these become centers of special activity in the single-celled organism the protoplasm of which is specialized or differentiated to this extent. Such centers, usually indicated by structural characteristics, by functional activity, or by susceptibility to certain dyes are *plastids* of the cell. Other substances in protoplasm by virtue of the reactions which they have undergone in the maelstrom of vitality become stabile, and no longer take an active part in the chemical and physical activities going on about them. Having gone beyond the plastic or labile state in metabolism, but carried along in the living protoplasm, they may serve a useful function in protection, support, offense, or defense of the organism. Such substances are called *metaplastids*.

C. Plastids of the Protozoa.—Centers of special activity, or plastids, are numerous and varied in Protozoa. Some, like chromatin, are present in all unicellular animals; others, like kinetic elements, are most conspicuous in actively moving forms. Some types like chromoplastids, pyrenoids and stigmata are associated with chlorophyll and autotrophic nutrition. Others like chromidia, volutin, and chondriosomes have obscure functions in the cell and are not yet fully proved to belong in the category of plastids.

1. **Chromatin.**—Chromatin is more a conception than a specific thing, the term being used to designate substances which appear under different forms at different phases of cell life. It appears normally in the form of minute granules or chromomeres (chromidiosomes of Minchin) in the resting nucleus, but during division of the nucleus these granules are massed together to form characteristic solid and individualized structures, the chromosomes. On *a priori* grounds chromosomes were early regarded as intimately associated with the phenomena of inheritance (Roux, Weismann, Boveri) and the more recent experimental work in genetics has given substantial evidence of the soundness of this early conclusion.

Our conception of chromatin is based largely upon investigations upon the nuclear substances of Metazoa and the higher plants. In ordinary descriptions, however, the term is often used in a vague sense to include any substance or body which stains with the so-called nuclear stains, *i. e.*, the basic anilin dyes, while direct chemical tests to determine the exact chemical composition of chromatin have been made in very few cases. The best of these show it to be composed of nuclein, one of the most complex of protein substances and rich in phosphorus.*

Vague as is the conception of chromatin in Metazoa it is even more so in connection with the Protozoa, where little has been done in

* For a critical discussion of chromatin, see Wilson, 1925.

a concrete way to throw light on the subject, although much has been written about it.

Many of the granules found in the cell body of a protozoön as well as those within the nucleus, stain with the usual nuclear dyes and their identification as chromatin is a matter requiring knowledge of their history and fate in the cell. It is only within recent years that an effort has been made to discriminate between the various granules in the Protozoa which stain intensely with the basic stains, and to distinguish the chromatin granules which enter into the make-up of chromosomes from other chromatoid granules which are distributed throughout the cell particularly the chromidia and the volutin grains. This is the more difficult in Protozoa because chromatin granules are not necessarily confined to the nucleus. Even in Metazoa and plants there are times during division when the chromatin is not confined within a nuclear membrane. In the Protozoa such a condition is permanent in many cases (*e. g.*, in Spirochetes, some flagellates, *Dileptus anser*, *Holosticha*, etc.). In other cases the nuclear chromatin, by transfusion or by nuclear fragmentation, spreads more or less widely throughout the cell protoplasm (rhizopods, *Actinosphærium eichhornii*, etc.). Here in different species, the fate of the distributed chromatin varies. In some cases this diffusion of chromatin indicates a degenerative change, the chromatin ultimately losing its characteristic reactions. Thus in *Actinosphærium eichhornii*, Hertwig has shown that, under adverse conditions such as starvation, or overfeeding, or during periods of depression, such distribution of the nuclear chromatin occurs, the granules ultimately becoming transformed into a characteristic pigment of the cell. In other cases the distributed granules retain their chromatin nature and according to numerous observers are ultimately aggregated into minute secondary nuclei which become the nuclei of conjugating gametes (many types of Rhizopoda and Foraminifera). In these instances, other chromatin which is retained in the "primary nucleus" takes no part in the germinal activities but degenerates and disappears after the gametes are liberated. It must not be inferred that germinal chromatin is thus distributed in the cytoplasm in all cases; on the contrary in the majority of Protozoa the gamete nuclei are derived by division of the morphological nucleus with its contained chromatin, and some authorities, notably Kofoid (1921) deny *in toto* the origin of gamete nuclei from chromidia.

While chromatin thus has a definite germinal function there is equally little doubt of its important participation in the ordinary metabolic activities of the cell. Thus, if an *Amœba proteus* or the ciliate *Uronychia transfuga* (see Fig. 108, p. 226), be cut into two portions one of which contains the nucleus while the other is enucleate, the former portion only will digest and assimilate food, grow

and regenerate the lost part, while the enucleate portion will continue to move and manifest various activities characteristic of destructive metabolism, but it will not take in food, nor digest what food may have been taken in before cutting and in the course of a week or ten days it dies (Hofer, Verworn, Balbiani and many others).

It is evident therefore that chromatin is directly associated with all of the important vital activities including reproduction, and the view has been repeatedly advanced that, for these varied activities at least, two different kinds of chromatin are responsible. One kind, the so-called vegetative or trophochromatin, is active in the ordinary metabolic functions of the cell, while the other, the germinal or idiochromatin, has to do solely with perpetuation of the race. While this view of the dual nature of chromatin would seem to be sustained by the phenomena in rhizopods and by the dimorphic nuclei in the ciliates, it is by no means assured that this duality represents a fundamental difference in chromatins. On the con trary it is much more probable, as Hertwig has maintained, that there is only one chromatin and that its functional activity depends upon different factors and conditions which may arise during the life cycle; germinal chromatin in one cell-generation may become vegetative chromatin in the next and *vice versa*. This is particularly clear in the case of the ciliates where the macronucleus, a distinctly vegetative nucleus, and the reproductive micronucleus, arise as subdivisions of a fertilization nucleus after conjugation or its equivalent parthenogenesis.

The importance of chromatin for life of the cell is indirectly indicated by the extreme precision with which it is distributed to daughter cells at the time of division. Like other granules of the cell each chromomere grows and reproduces its exact duplicate by division. Chemically it probably represents the pinnacle of complex structures formed as a result of the activities of constructive metabolism while its derivatives, likewise granular in form and difficult to distinguish as such, formed by reductions, deamidizations and other chemical processes, give rise to many more or less permanent or temporary structures in the cell body, each of which may perform some cellular activity in its passage through the various stages of disintegration.

2. **Chromidia.**—As stated above, chromidia are only chromatin granules distributed in the cytoplasm, and the main significance in the term as we use it today is to indicate the extra-nuclear position of chromomeres. They have come to be regarded as characteristic structures of the protozoön cell, however, and students of the Protozoa speak of chromidia with the same familiar ease that they do of the nucleus. In some types a definite nucleus is entirely absent and such forms provide the only justification for Haeckel's

hypothetical group of Monera. In the majority of Bacteria and spirochetes and in more complex ciliate types like *Dileptus anser*, *Holosticha*, etc., the functions which the cell nucleus are supposed to perform are either absent altogether, or, which is more probable, they are performed by the distributed chromidiosomes or chromidia.

3. **Volutin Grains.**—These are widely distributed in Protozoa and are difficult to distinguish from chromidiosomes. They are usually spherical in form and stain intensely with the basic dyes, retaining the stain even after the chromatin granules are completely extracted. They were discovered by a pupil of A. Meyer in the cells of *Spirillum volutans* from which the peculiar name is derived, and, according to Guilliermond, they are identical with the "metachromatic bodies" of Babes, and with the "red granules" discovered by Bütschli. Meyer regarded them as composed largely of nucleic acid, a conclusion supported by the experiments of Reichenow (1909) on *Hematococcus* in which it was shown that volutin grains disappear in a medium free from phosphorus and that, during the phases of active chromatic increase in the nucleus, they diminish perceptibly in size and increase in size when the chromatin content becomes stationary. From these results, confirmed by van Herwerden (1917) on yeast cells, Reichenow concluded that volutin grains play a most important part in the vital activities of the cell and he regards them as a reserve store of nucleo-proteins for the purpose of chromatin growth in the nucleus. They take a yellow stain with iodine and a red stain with methylene blue and 1 per cent solution of sulphuric acid, while their reaction to the usual chromatin stains makes them difficult to distinguish from chromidia. They appear to be formed in the cytoplasm and, if these observations are well founded, are entirely different in origin and in function from the other minute granules which they closely resemble. The importance of these conclusions in problems connected with biology of the cell warrants the demand for further and more complete observations and experiments.

4. **Chondriosomes** (*Mitochondria*).— Chondriosomes appear to be permanent granules in the cytoplasm of many types of Protozoa in which they have been studied mainly by Fauré-Fremiet, Vonwiller (1918), and Cowdry (1918). Like other granules in the cytoplasm they are usually spherical and very minute (0.5 μ to 1.5 μ in diameter), but unlike many other granules each appears to retain its individuality from generation to generation by dividing prior to or during division of the cell.

Observations of the chondriosomes of Protozoa are too scanty to permit of definite conclusions regarding their history or function in the cell and their chemical composition is quite unknown. Fauré-Fremiet regards them as combinations of albumin with phosphates or with fatty acids, and believes that they play a part in con-

nection with the preparations for sexual activities of the organism, a very general conclusion which, so far as it goes, appears to be justified by the rapidly accumulating facts concerning chondriosomes in the sex cells of Metazoa.

5. **Chromoplastids and Pyrenoids.**—Other permanent cytoplasmic structures of the Protozoa are the color-bearing bodies termed chromoplastids, chloroplastids or chromatophores, and the pyrenoids, both of which are characteristic of the autotrophic forms, whose nutrition is dependent upon photosynthesis (see Chapter IV). They are found mainly in the group of flagellates although an occasional form in other groups of Protozoa may contain them (*e. g.*, *Paulinella chromatophora, Chlamydomyxa montana,* amongst rhizopods). Pyrenoids usually accompany and are embedded in the chromoplasts.

Chromatophores vary greatly in form and size; spherical, discoidal, band-form, ring-form, spindle-form and irregular types are known, while the number in a single organism may vary from 1 to 100 or more. They invariably increase by division and are to be regarded as permanent organoids of the cell. Division, however, may in some cases at least be quite independent of the division of nucleus or cell body. The pyrenoids are usually spherical and are characteristically refractile structures either single in the cell or as numerous as the chromoplastids. Like the latter they also may reproduce by division.

The ability to create different colored substances included generally under the heading chromophyll is the chief characteristics of chromoplastids. The colors vary from the typical plant green chlorophyll, of various shades of green (Phytomonadidæ, Chloromastigidæ), through brown and yellow colors (Chrysomonadidæ), blue-green (*Paulinella chromatophora*), and red (hematochrome). In the majority of forms the coloring matter appears to be identical with the chlorophyll of higher plants; the yellow colors, especially that of the Dinoflagellata, resembles the coloring matter (diatomin) of the diatoms, while the red color, hematochrome, is a modification brought about apparently by the diminution of nitrogen and phosphorus in the surrounding medium (see Reichenow, 1909).

Chromatophores are not to be confused with the symbiotic algæ which live normally in the protoplasm of many kinds of Protozoa (*e. g.*, "yellow cells" of Radiolaria, Zoöchlorellæ of *Paramecium bursaria,* etc.). In all cases the green chlorophyll is readily transformed, as in higher plants, into yellow xanthophyll, and the red hematochrome into green chlorophyll by treatment with dilute alcohol.

D. Metaplastids of the Protozoa.—In the protoplasm of all Protozoa, in addition to the permanent granules of one kind or another described above, there are many types of transitory or

fixed products of cell activity collectively known as metaplasmic granules or metaplastids. All of these are formed during the vital activities of metabolism some of them as reserve stores of food substance formed as products of the building up or anabolic processes of metabolism, others by the destructive or catabolic processes. In the former group are included many kinds of carbohydrates such as amylum (starch); paramylum (similar in composition to starch but fails to give the characteristic blue reaction with iodine), karotin, leucosin and cellulose, all of which are characteristic of chlorophyll-bearing Protozoa although not confined exclusively to them; other products of constructive metabolism which are more widely distributed, are fats, glycogen, paraglycogen, oils, albumin spheres, etc. In the latter group, as products of destructive metabolism, are included a great variety of crystals, pigment granules, chitin and pseudo-chitin, and other more or less widely distributed products. These products of destructive metabolic activities are frequently so abundant as to give the protoplasm a densely granular appearance.

The form and appearance of these various products of protoplasmic activities vary within wide limits and will be discussed more fully in connection with the different classes of Protozoa. Many of them serve a useful purpose as reserves in nutrition and other physiological processes, while a number of them are used for purposes of support, protection, or shell and skeleton building. Carbohydrate compounds, rarely in the form of starch, but abundantly in the form of paramylum, are mainly confined to the chlorophyll-bearing Protozoa where, in forms like *Euglena* they are the first recognizable products of assimilation. After their formation they may remain as a reserve store of nutriment. True starch occurs in the Cryptomonadidæ, Phytomonadidæ, and in the Dinoflagellata, while paramylum may occur, not only in the chlorophyll-bearing types, but in many colorless forms as well (*e. g. Chilomonas paramecium, Astasia, Peranema,* etc.). Glycogen-like bodies are found in a few types of flagellates; true glycogen occurring in the protoplasm of *Pelomyxa palustris* according to Stolç (1900), and in the ciliates *Paramecium, Opalina, Glaucoma* and *Vorticella* according to Barfurth. Paraglycogen, also called zoöamylum, which differs from glycogen in its solubility and in its color reactions when subjected to sulphuric acid and iodine, is present in many ciliates and flagellates as well as in some gregarines. Leucosin is a carbohydrate in the form of highly refractile globules or balls particularly characteristic of the Chrysoflagellidæ and some of the simpler Monadidæ.

Oils and fats are widely distributed. Great oil globules are particularly characteristics of the Radiolaria where, in addition to serving a useful purpose as reserves of nutriment, they also serve

a hydrostatic function in the activities of different organisms. Globules of smaller size but conspicuous by their frequently brilliant coloring are found in many types of flagellates and ciliates. In some cases, notably in *Noctiluca miliaris* and in several Dinoflagellates, these metaplasmic oils are photogenic and, in contact with oxygen, produce phosphorescence often of great brilliancy (de Quatrefages, 1850, E. B. Harvey, 1917). Similar globules of oil stored up in flagellated Protozoa, minute as they are in the individual, may become a great nuisance collectively. Potable waters, for example, are frequently rendered unpalatable because of the odors and tastes due to these products of metabolic activity. Such objectionable odors and tastes are rarely due to the putrefaction of the organisms, but rather to the liberation of minute drops of oil upon disintegration of the cell bodies. As crushing a geranium leaf causes minute drops of oil to be thrown in the air, giving the fragrant perfume of that plant, so disintegration of cells of *Uroglenopsis americana*, crushed by the pressure in pumps and mains, causes the liberation of minute oil drops stored in the protoplasm, but the cod-liver oil smell which they impart to the water is far from fragrant. So characteristic are these metaplasmic products of the organisms which produce them that many kinds of flagellates which accumulate in drinking waters may be recognized simply by the odors which they impart (Calkins, 1891).

Cellulose and pseudo-chitin are products of cellular activity which are useful in the formation of membranes, shells and tests. Cellulose, as in higher plant cells, forms the lifeless membrane of many chlorophyll-bearing types, while protein derivatives in the form of chitin and pseudo-chitin are more widely distributed through the entire group of Protozoa, forming the substratum upon which, or between layers of which, shell materials are deposited, while cups, tests or "houses," cyst membranes, stalks etc., are formed directly from its substance. Shell and skeleton materials such as calcium carbonate, silica, strontium sulphate, etc., are likewise formed as results of metabolic activity, sometimes continuously, as in the limestone shells of the Foraminifera, and sometimes periodically at intervals of saturation (dictyotic or lorication moment) as in the formation of the characteristic silicious skeletons of the Radiolaria.

Pigments of various hues are also frequently found in Protozoa. In some cases, as in *Actinosphærium eichhornii*, they are formed as a final product of degeneration of chromatin granules (chromidia); in other cases they are products of metabolic activities following the digestion of specific kinds of food, as melanin pigment, brown or black in color, which follows the digestion of hæmoglobin by malaria-causing hemosporidia (*Plasmodium* species). Specific coloring matters are found here and there, especially amongst the ciliates which have nothing to do with chlorophyll and which are named

according to the organism in which they are found. Thus the blue coloring matter sometimes called stentorin, is characteristic of *Stentor cœruleus* and some species of *Folliculina;* a red pigment of *Mesodinium rubrum;* violet of *Blepharisma undulans,* etc.; the colors

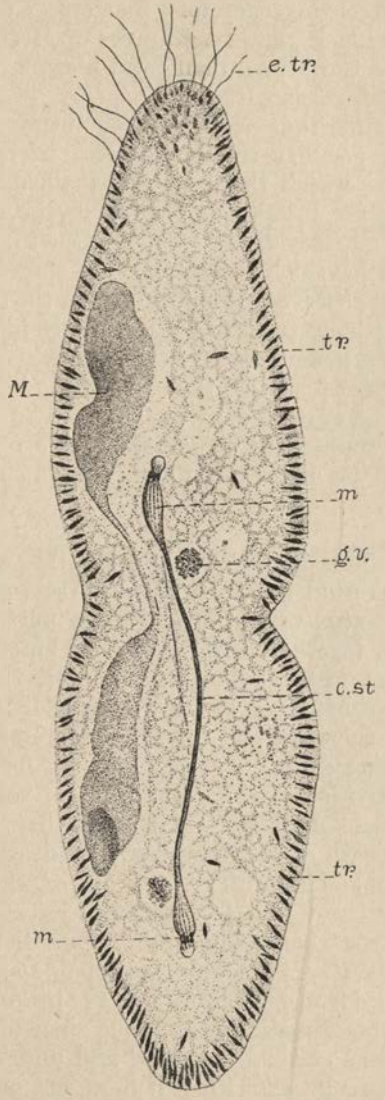

FIG. 21.—*Paramecium caudatum.* Section of a dividing individual; *c. st.*, connecting strand of dividing micronuclei; *e. tr.*, extruded trichocysts; *g. v.*, gastric vacuole; *M*, dividing macronucleus; *m, m*, divided micronuclei; *tr.*, trichocysts. (Original..)

being due, probably, to the kind of food that is eaten, since the pigmentation of the same species is not constant, some forms in the same culture of *Blepharisma undulans*, for example, may be colorless while others are more or less bright pink, or violet, or even purple in color. In many cases the pigment is accumulated in masses of varying size representing excretory matters of one kind or other. Thus we find the black pigment granules of *Metopus sigmoides* and *Tillina magna*, or the brown pigmental masses (phæodium), characteristic of the tripylarian Radiolaria.

Other metaplastids that are useful for purposes of protection or support, are the peculiar trichocysts and trichites found in the ciliates and about which there is very little definite information (Fig. 21). They are usually embedded in the cortex when fully formed but the trichocysts at least appear to be formed in the vicinity of the nucleus as Mitrophanow has shown for Paramecium, and as I have also observed (unpublished) in the case of *Actinobolus radians*. The trichocysts at rest are capsules filled with a densely staining (with iron hematoxylin) substance which is thrown out in the form of long threads when the organisms are violently irritated as with poisons of one kind or another. The trichites are stiff, usually rod-like supporting structures and are rarely discharged (for discussion of the distribution and functions of these structures see Chapter III).

All of the structures described above, together with the defecatory materials such as sand grains, shells and tests of other organisms taken in as food, etc., which never form a part of the living substance, make up, together with nuclei and kinetic elements which will be considered in the following Chapter, the granular aggregate of colloidal substances which we see in living protozoön protoplasm. Their chemical and physical reactions and interactions, in abeyance during encystment, combine to furnish the manifold physiological activities of the organism and to distinguish living things from lifeless matter. Their possibility of living activity ends only with death of the organism, and death, one of the most remarkable phenomena of life, is the disintegration of the protoplasmic organization which forms the physical basis of vitality. Accepting the view that spontaneous generation of living things under present conditions is highly improbable and for which there is no acceptable evidence, it follows that the protoplasm of all things living today has been continuously living since life appeared on the earth. How it was originally formed and under what conditions, are matters of speculation with which we are not concerned here.

Protozoa, finally, should be regarded as single-celled organisms notwithstanding the views of Whitman *et al.* concerning the inadequacy of the cell theory as interpreted by Whitman, Gurwitsch, or

Hartmann. There is not much to be gained by the substitution of the "energid" theory of Sachs, Strasburger, and Hartmann. If necessary the conception of the cell should be expanded to permit of the inclusion of all Protozoa with their varied intracellular differentiations and with their invariable performance of all of the fundamental vital activities included in the physiological attributes of higher animals and plants. Each of them is a perfect organism and some of them, in a morphological sense, represent most extreme types of intracellular differentiation, although not in the sense of cell specialization and functional limitation.

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CHAPTER II.

NUCLEI AND KINETIC ELEMENTS.

In the preceding chapter plastids in protoplasm were interpreted as substances of more or less homogeneous nature which act as centers of specific activities or activity. Kinetic elements of the cell might well be included in this category of plastids since they are apparently composed of homogeneous substance and have specific activities in connection with the visible expressions of the transformation of energy through destructive metabolism. Nuclei, on the other hand, are not homogeneous substances, but are aggregates of substances of different kinds and amongst these substances are some which are unmistakably kinetic in function. These aggregates of substances are the centers of a great variety of activities in the cell, the importance of which is evident by the simple experiment of cutting a cell so that one fragment contains the nucleus, while the other fragment has none. The enucleated fragment is unable to digest and assimilate food or to grow; nor is it able to reproduce, nor to regenerate lost parts except under certain circumstances (see p. 485). Nucleated fragments on the other hand are able to do all of these and continue to live as normal organisms.

Chomatin and kinetic elements are closely associated in protozoan nuclei and no adequate discussion of either is possible without a discussion of both. Here the nucleus is not only the site of chromatin aggregates but there is abundant evidence, and further evidence is constantly accruing, to support the view that the nucleus is the original seat of kinetic elements as well. The well-known views of Schaudinn (1904), the oft-repeated statements of Hartmann and of Kofoid, and the conclusions of Jollos (1917), of Belar (1920) and others, all agree in regarding the nucleus of a protozoön as a combination of kinetic and idiogenerative elements.

I. THE NUCLEI OF PROTOZOA.

The term "nucleus" is ordinarily applied in a morphological rather than a physiological sense. If the activities of the component parts of the nucleus are absolutely necessary for the maintenance of life of the cell, then, in some cases such as *Holosticha*, *Trachelocerca*, or *Dileptus anser*, such activities must be performed by substances which appear to be identical with chromatin but which are distributed throughout the cell. On the other hand, it is highly prob-

able that some functions are possible by virtue of the physical properties of a definite, but permeable, nuclear membrane, and as in the tissue cells of Metazoa, it is this type of membrane-bound nucleus that we find in the vast majority of Protozoa.

In their resting stages the nuclei of Protozoa present a bewildering variety of forms and structures, differing in this respect from the

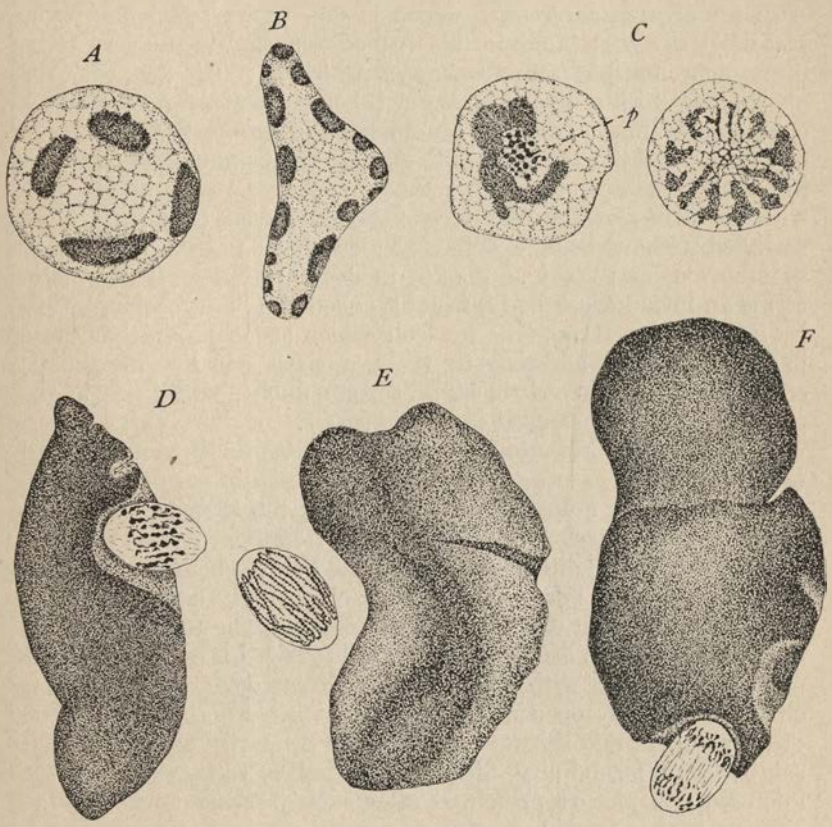

FIG. 22.—Types of vesicular and massive nuclei. *A*, vesicular type of *Pelomyxa binucleata; B*, of *Polystomella crispa;* both with multiple endosomes; *C*, nucleus of *Actinosphærium eichhornii* with granular plastin (*p*); *D*, *E*, *F*, macro- and micronuclei of *Paramecium caudatum*, the latter in different stages of vegetative mitosis. (*A*, *B*, after Doflein; *C*, after Hertwig; *D*, *E* and *F*., original.)

much less variable tissue nuclei of the Metazoa. Because of these manifold differences students of the Protozoa have experienced great difficulty in grouping nuclei for purposes of description. They agree, however, in recognizing two primary nuclear types, the *vesicular* and the *massive*. Nuclei of the massive type more clearly resemble the nuclei of spermatozoa being filled with small chromatin

granules, but they rarely present the homogeneous appearance of a spermatozoön nucleus, the individual granules, although closely packed, being recognizable (Fig. 22).

Certain constantly recurring substances are characteristic of protozoan as of metazoan nuclei, but some types of arrangement and combination of these substances are typical of Protozoa and are rarely found in Metazoa. The most universal of these nuclear constituents are (1) chromatin, which is sometimes called nuclein or identified as such; (2) linin, also called achromatin, nuclear reticulum, achromatinic framework, etc., which is continuous with the alveolar network of the cytoplasm; (3) nuclear membrane which is composed of linin, and forms a permeable partition or wall between cytoplasm and nucleoplasm; (4) nuclear sap or nuclear enchylema filling the spaces of the linin reticulum; this seems to be identical with the intra-alveolar substance of the cytoplasm; (5) plastin, often so-called without being specifically identified as such; also termed paranuclein, or pyrenin. Plastin in combination with chromatin forms an intranuclear body, usually called the "karyosome," while by itself plastin forms true nucleoli which are comparatively rare in Protozoa. In addition to these a sixth constituent, kinetic elements are characteristic of protozoan nuclei, and these in the present work will be called *endobasal bodies*.

It must be frankly admitted that very little is known in regard to the chemical nature of these various constituents of the nuclei in Protozoa and much confusion exists in the literature owing to the promiscuous use of these terms in relation to structural elements of the nucleus without knowledge of the actual chemical make-up.

(*a*) **Chromatin.**— Few investigations of a purely chemical nature have been made on chromatin of Protozoa. The usual procedure is to designate as chromatin all structures of the nucleus or cytoplasm which stain with the so-called nuclear dyes, or to interpret chromatin mainly on a morphological basis. Microchemical tests of all protoplasmic substances are made primarily on the basis of solubility or insolubility with acids, alkalies, salts, etc., and the microscopical picture presented after such treatment leads to the conclusion that certain structures are made up of certain substances. Such tests do not prove that a given structure is composed of a definite substance and is not a mixture of substances, but they are useful in the main to indicate that different structures are essentially different in chemical make-up even though the exact chemical composition remains a secret. Kossel, Miescher and others have shown that the chromatin bodies composed of the chemical substance nuclein are not dissolved under the action of artificial gastric juice (pepsin and trypsin in appropriate acid and alkaline media) while other portions of the nucleus such as nucleoli, reticulum and plastin are entirely dissolved. On the other hand chromatin bodies are

dissolved in strong acids, dilute alkalies, calcium carbonate and sodium phosphate some of which have no apparent effect on nucleoli which remain undissolved in potassium hydrate or in a 1 to 3 per cent acetic acid (Doflein).

That there is a great difference, however, in the ultimate chemical composition of the nuclear structures which we call chromatin is apparent, as Minchin clearly points out, from the diversity of forms of life, although the chromatin contained in them appears to be the same. If chromatin is the seat of factors having to do with definite adult structures it follows that chromatin in different organisms must be different in ultimate composition. Or, to state it in another way, the differences between *Amœba*, sea-urchin and mammal are relatively no greater than the differences between *Amœba*, the egg of a sea-urchin and the egg of a mammal, nor are these relatively greater than the differences between the chromatin of *Amœba*, of the sea-urchin and of the mammal. Chromatin in these three cases represents the last stage of evolution in each no less surely than the adult structures do, but they are beyond reach with our present means of analysis.

In vesicular nuclei the chromatin granules may be distributed more or less evenly throughout the nucleus, or they may be segregated in "net-knots" or either alone or combined with other nuclear substances in one large central globular mass to which Minchin gives the name *endosome* as an equivalent for the term *Binnenkorper*, or they may be aggregated in several such globular masses or multiple endosomes distributed throughout the nucleus or plastered to the nuclear membrane.

Endosomes may consist entirely of chromatin as appears to be the case in nuclei of some Microsporidia (*Glugea* and *Thelohania*), or some flagellates (*Prowazekia*, Belar, 1920, etc.), or in the multiple endosomes of *Noctiluca miliaris* or of *Polystomella crispa*. Or they may be composed of chromatin and plastin in various combinations. Thus in *Actinosphærium eichhornii* in some stages of nuclear activity, the chromatin component is in the form of an incomplete ring which partially encloses the plastin portion (Fig. 22, c). In other cases the plastin is entirely surrounded by a cortex of chromatin which may be dense and compact as in the case of many types of rhizopods and Sporozoa or loosely aggregated as in nuclei of *Endamœba intestinalis* (Fig. 23). The distributed granules of deeply staining material which represent the substitute for a nucleus in *Dileptus anser* are similarly composed of a plastin core and a chromatin cortex, the former increasing enormously after treatment of the animal with certain kinds of food such as beef broth. Here the term endosome is scarcely applicable since the bodies in question are not inside a nuclear membrane, but they appear to be morphologically equivalent to these intranuclear

structures. After treatment with beef broth the body of *Dileptus* is enormously distended due to the swelling of these cytoendosomes (Fig. 24).

The centrally placed intranuclear body is generally described under the name *karyosome*, a term which has been so widely used by students of the Protozoa and for so many obviously different structures that it is practically synonymous with endosome or Binnenkorper. Thus Minchin describes it as a combination of chromatin and plastin; Doflein defines a karyosome as a centrally placed, sharply outlined and constant constituent of the nucleus, which may contain no chromatin or may be a combination of other substances with chromatin and which divides during nuclear division, to form

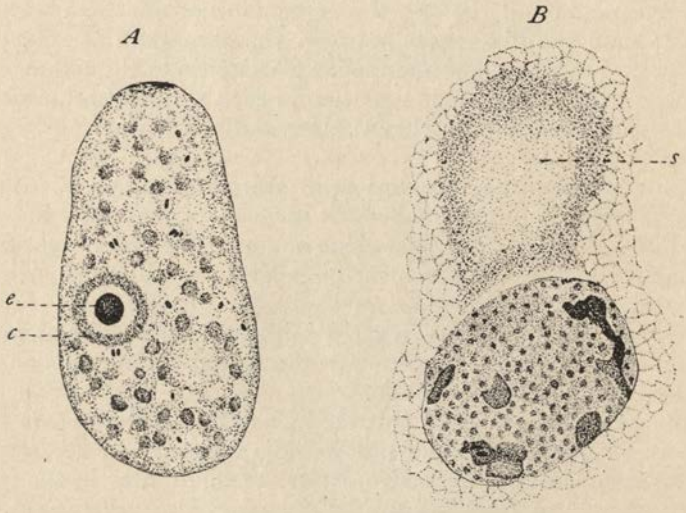

FIG. 23.—*A*, *Endamœba intestinalis*; (*e*) endosome; (*c*) cortex of chromatin; *B*, nucleus and "sphere" (*s*) of *Noctiluca miliaris* with multiple endosomes. (Original.)

two corresponding daughter structures (Doflein, 1916, p. 22). Hartmann's (1911) definition is more limited, a karyosome in his use of the term being an endosome (Binnenkorper) containing a centriole. Belar (1921) finds a "karyosome" in *Chlamydophrys minor* which breaks up and disappears forming neither chromatin nor kinetic elements. If we attempt to combine these different views into a common definition we find that a karyosome may be an intranuclear body which may consist of plastin alone; or kinetic element alone; or chromatin together with plastin; or a combination of chromatin with kinetic elements; or a combination of chromatin, plastin, and kinetic elements. Such a definition obviously would fail to specify any particularly nuclear structure and so far as its

practical value is concerned the term karyosome is no more useful than the non-committal term Binnenkorper or Minchin's equivalent term endosome. I would advocate, therefore, discarding altogether

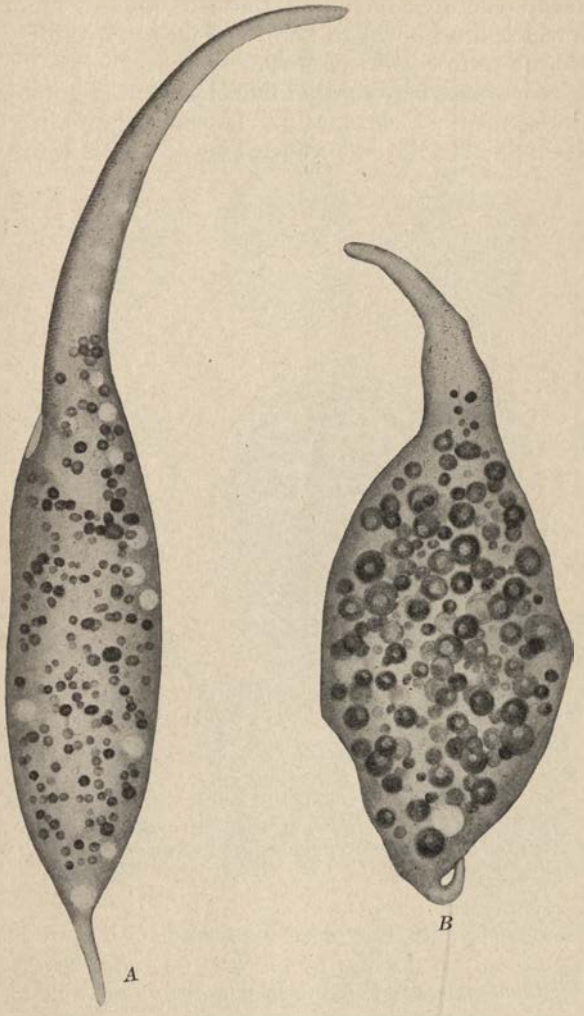

FIG. 24.—*Dileptus anser: A*, vegetative individual in culture with nucleus in the form of scattered chromatin granules; *B*, individual showing the effect of treatment with beef extract on the chromatin granules. (Original.)

the term karyosome which seemingly bears the earmarks of something definite in the cell, using in its place the general non-committal expression Binnenkorper, or its equivalent term endosome, the

latter as yet, at least, having no specific significance, while for the endosomes having functions characteristic of the kinetic complex a specific term may well be applied. In the present work I shall employ the term endosome in a general way to indicate all central intranuclear structures including those of kinetic function, while for those which are known to be of the nature of kinetic elements I shall use the term *endobasal body*.

The endosome-bearing vesicular nuclei present manifold variations in the arrangement of chromatin. In some the entire chromatin content is confined to the endosome which seems to rest in the center

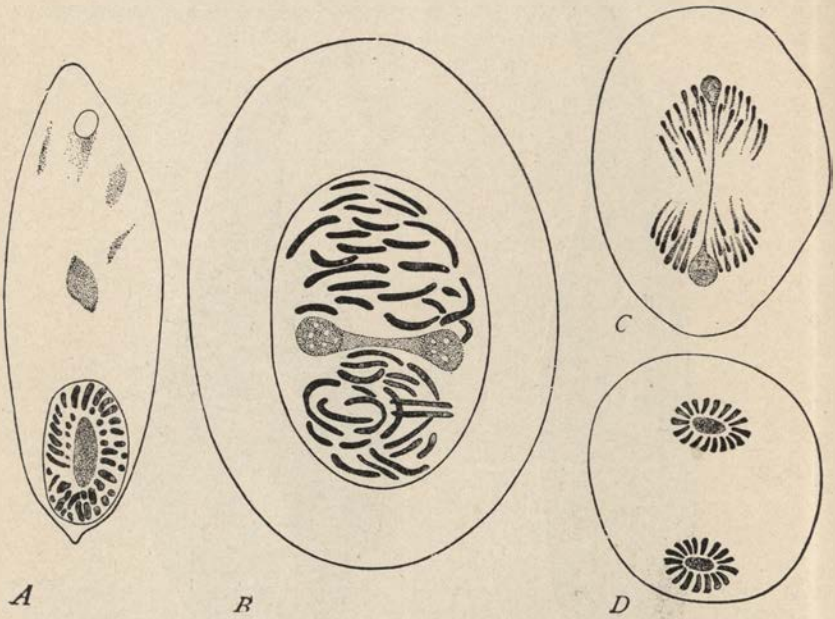

FIG. 25.—Division in *Euglena viridis;* nucleus with endobasal body. *A*, preparing for division, the endobasal body surrounded by "chromosomes;" *B*, *C* and *D*, successive stages in nuclear division. (From Wilson after Keuten.)

of a colorless enchylema traversed by strands of linin radiating from the endosome to the nuclear membrane (*Arcella vulgaris, Cochliopodium bilimbosum* and rhizopods generally, as well as in many Coccidia and Gregarinida). In other cases the endosome retains only a little of the chromatin, the bulk of which is present as a dense network in the zone between endosome and membrane (*Amœba intestinalis, A. crystalligera*, etc.). In still other cases the chromomeres are distributed more or less uniformly throughout the nuclear reticulum (*Euglypha alveolata*, etc.).

In vesicular nuclei with endobasal bodies the chromatin may be

in the form of more or less regular chromomeres uniformly distributed in the nuclear space (*Euglena* type, Fig. 25), or more or less compactly aggregated about the kinetic element (many species of *Endamœba*, various flagellates, Coccidia and Myxosporidia, etc.). Or, finally, the chromatin may be in the form of relatively large granules collected in a zone just within the nuclear membrane (*e. g., Pelomyxa*), or in fine granular form may make up the chief part of the nuclear membrane (*Vahlkampfia limax,* Fig. 26).

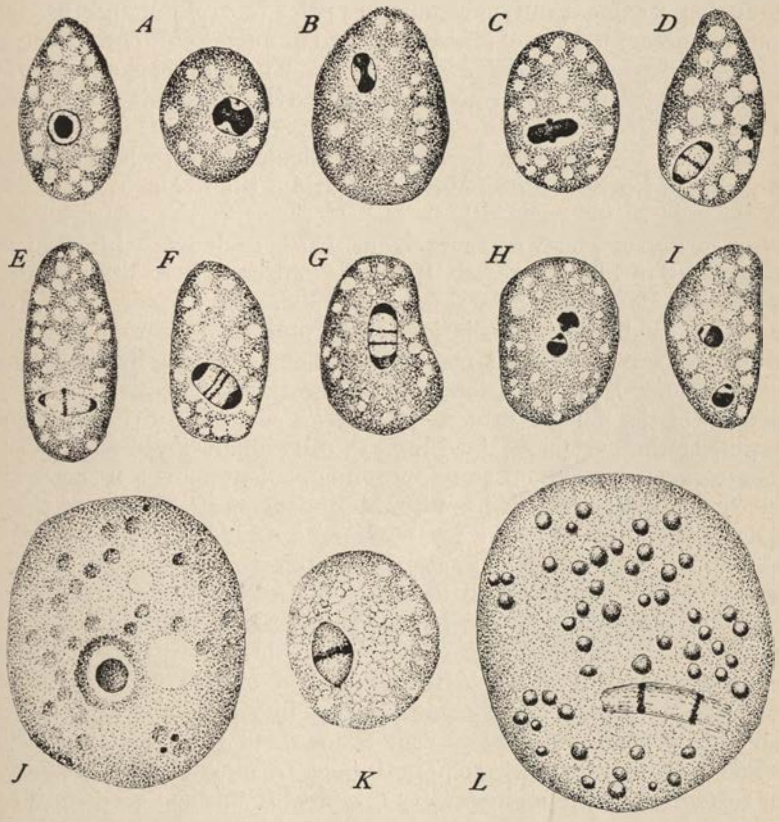

FIG. 26.—Division of amœbæ. *A* to *I*, successive stages in division (promitosis) of *Vahlkampfia limax; J* to *L*, mitosis in *Endamœba coli.* (Original.)

A peculiar and most unusual type of vesicular nucleus is present in *Noctiluca miliaris* and has the superficial appearance of a massive nucleus. Two distinct types of structure have been described, one by Doflein, the other by Calkins, and the descriptions differ so widely that it is difficult to recognize them as pertaining to the same organism. According to Doflein, the nucleus belongs to the massive

type having uniform chromatin granules distributed upon a regular reticulum and with nucleoli of irregular form and size throughout its substance. According to Calkins there is no evidence of a chromatin reticulum, but the massive character of the nucleus is due to the presence of relatively large and stiff colloidal masses of intra-alveolar material while the chromatin is aggregated in the form of ten or eleven irregularly distributed masses which were called "chromatin reservoirs" but which appear to be multiple endosomes. That these masses consist of chromatin is clearly indicated by the fact that they fragment into chromomeres prior to division and that the chromomeres form the characteristic and unmistakable chromosomes of the nuclear spindle (Fig. 52, p. 101). These diverse accounts of the nuclear structure of *Noctiluca* indicate the possibility of regional varieties of this universally distributed species.

Mention may be made here of the vesicular nuclei which arise by a process of so-called free-nuclei formation, the evidence for which is difficult to interpret otherwise. It rests, in the main, on the observation of Hertwig as early as 1876, and again in 1899; of Schaudinn in 1903; of Lister, 1905; of Goldschmidt in 1907; Elpatiewsky in 1907, and Swarzewski in 1908. In all cases the free nuclei arise by the association of chromidia or chromidiosomes which have been derived from the nucleus and distributed in the cytoplasm. Both Elpatiewsky and Swarzewski describe the formation of the minute gametes of *Arcella vulgaris* by the fragmentation of the cytoplasm into minute cells about these free nuclei. These gametes move off as minute amœbæ leaving the parent with its "primary" nuclei, which ultimately degenerate. Each of these gametes contains at first a few scattered granules derived from the chromidial mass which ultimately unite to form the gamete nucleus. The process is more minutely described by Goldschmidt in connection with the mastigamœba *Mastigella vitrea*. Here a chromidial mass forms on the outside of the nuclear membrane by transfusion of chromomeres (Fig. 27). After separation of this mass from the nucleus, the chromomeres come together in groups and form nuclei about which minute gamete cells are cut out from the cytoplasm while the primary nucleus remains intact. A somewhat similar mode of formation of the microgamete nuclei of *Coccidium schubergi* was earlier described by Schaudinn. This type of nucleus formation, according to Minchin, represents the possible origin of Protozoa of "cellular grade" from bacteria-like organisms of non-cellular grade, in which the chromatin is permanently distributed. Doflein (1916) remains skeptical in regard to this type of free-nuclei formation and Kofoid (1921), apparently without investigation of free-living forms, maintains that such free nuclei are intracellular parasites. It is evident that the burden of proof here rests with the critics.

In the massive type of nucleus the chromidiosomes are usually of

similar size and are densely packed throughout the entire nucleus, giving a characteristic appearance after staining. They are widely distributed in Dinoflagellata, Ciliata and Suctoria, but there is considerable variation in their density in different species, especially in the Infusoria. In some of the micronuclei (*e. g.*, *Paramecium caudatum*, *Euplotes patella*, etc.), the chromidiosomes are so tightly packed as to give them, more than any other type of protozoön nucleus, the aspect of a spermatozoön head (Fig. 22, *D*, *E*, *F*). In other cases the granules are very fine and follow the course of the linin network thus affording an excellent picture of the alveolar structure within the nucleus.

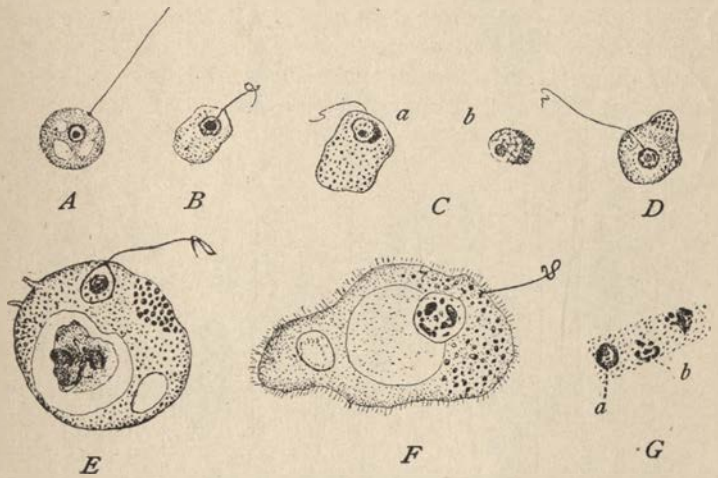

FIG. 27.—Chromidia formation in *Mastigella* and *Mastigina*. *A*, *B*, young forms of *Mastigella vitrea* prior to chromidia formation; *C*, chromidia arising from the nucleus *D*, young form of *Mastigina setosa* with accumulation of chromidia; *E*, *F*, mature stages of *M. setosa; G*, formation of gametic nuclei (*a*) from scattered chromidia. (After Goldschmidt.)

The formation of the massive type of nucleus during reorganization after conjugation is clearly shown in the case of *Uroleptus mobilis* (Fig. 1, Frontispiece). The young macronucleus is formed by a second division of a fertilization nucleus after conjugation when it appears as a vesicular nucleus with a fine linin reticulum which has no staining capacity. In life it appears like a large, highly refractile, vacuole. It remains in this ghost-like condition for a period of three or four days, enlarging meanwhile and becoming ellipsoidal in form. Chromatin ultimately makes its appearance in the form of minute granules on the nuclear reticulum. These granules increase in number and in size until the characteristic dense nucleus with intense staining capacity results (Fig. 28). It then divides with the

first post-fertilization division of the cell, and each daughter-nucleus divides three times.

(*b*) **Linin.**—The achromatic reticulum of the nucleus appears to be continuous with the alveolar reticulum of the cytoplasm, the

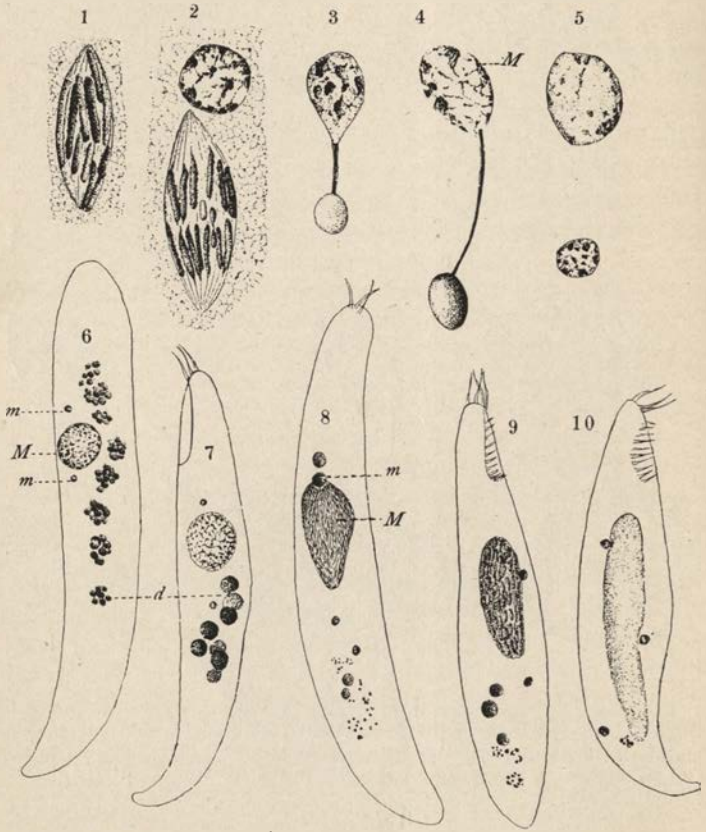

FIG. 28.—Origin of macronucleus after conjugation in *Uroleptus mobilis*. (1) First metagamic mitosis of the amphinucleus; (2) one of the progeny of this division dividing again; (3), (4), (5) telophase stages of second division of the amphinucleus resulting in a new macronucleus (above), and a degenerating nucleus (below); (6 to 10), stages in differentiation of the young macronucleus and disintegration and absorption of the old macronucleus; in (10) two new micronuclei are in mitosis preparatory to the first division of the ex-conjugant. (*M*) new macronucleus; (*m*) new micronuclei; (*d*) degenerating old macronuclei. (After Calkins.)

continuity of protoplasmic stuffs being unbroken in the living organism. In fixed and stained cells, however, unless the fixation is perfect, there is very apt to be a clear space between the nuclear membrane and the cytoplasm. Such perinuclear spaces are due to the shrinkage accompanying coagulation of the colloidal proto-

plasmic substances under the action of the killing fluids and are always to be interpreted as artefacts. The chromidiosomes are suspended in, and held in place by, the linin reticulum, which in some cases is extremely delicate and difficult to see, while the interalveolar spaces are filled with fluid enchylema (*e. g., Arcella vulgaris*). In other cases, owing to the suspension of very fine chromomeres, the outlines of the intranuclear alveoli are characteristically distinct (*e. g., Acineta grandis*). In most massive nuclei, however, the comparatively large chromidiosomes distort the alveolar walls, more or less completely obliterating the reticulum.

(*c*) **Membrane.**— Like other constituent parts of the protozoön nuclei, the membranes are highly variable, sometimes presenting in optical section only one contour on the outer side (*e. g., Actinosphærium*); sometimes showing contours both outside and inside (*Amœba proteus*). In the former case the inner zone adjacent to the membrane shows a decreasing density inwards, until the linin merges insensibly into the intranuclear reticulum. In free-nuclei

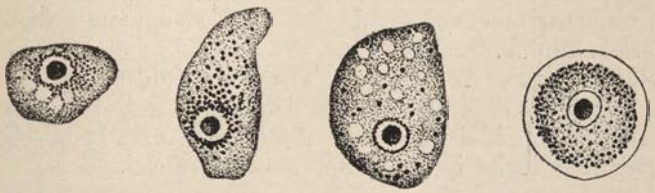

FIG. 29.—*Vahlkampfia limax;* chromatin forming the nuclear membrane and giving rise to chromidia. (After Calkins.)

formation, antecedent to gamete formation described above, the nuclear membranes are probably formed from the cytoplasmic reticulum in which the chromidiosomes are lying. Chromomeres also take part in the formation of nuclear membranes in some cases, *e. g.*, in *Vahlkampfia limax*, where the linin membrane is too delicate to be seen, although the definite limitation of the chromomeres indicates its presence (Fig. 29).

One peculiarity of the nuclear membranes of Protozoa which distinguishes them from nuclear membranes of tissue nuclei, is that in the majority of cases they remain intact during all phases of cellular activity and only rarely disappear, or disappear in part only, during division processes of the cell. (For description of chromatin, membranes etc., during division, see p. 114.)

(*d*) **Plastin.**— Plastin, perhaps not different from linin, has been definitely identified only in a few cases of Protozoa, and much remains to be done before an accurate account of its functions in the nucleus can be written. Probably a derivative of chromatin as early suggested by van Beneden, it is an important substance in

the make-up of the all types of nuclei, occurring in pure form in the nucleoli of tissue cells, but only rarely as such in Protozoa. Although rare in this pure form (Reichenow describes it in the nucleus of *Hæmogregarina stepanowi*), it is widely distributed in combination with chromatin, and the majority of endosomes are made up largely of plastin. From such combinations the plastin or the chromatin constituent may be separated out during nuclear division or during certain other phases of cellular activity. Thus Hertwig has shown that the endosome of *Actinosphærium eichhornii*, consisting of chromatin and plastin in rather loose combination, loses its chromatin which is then distributed over the nuclear reticulum while the erstwhile plastin endosome becomes a true plastin nucleolus with characteristic reticular structure and staining capacity (Fig. 22, c). Plastin, furthermore, appears to be the ground substance by which chromidiosomes are cemented together to form compact chromosomes during division, or to form a more or less definite mass of cytoplasmic chromidia (see Goldschmidt, 1906, *Mastigella vitrea*). It also appears to be associated with, and to form the matrix of, kinetic elements or endobasal bodies and selects the acid dyes in differential staining.

(*e*) **Nuclear Sap or Enchylema.**— The more fluid substance of the nucleus appears to differ in no marked degree from the intra-alveolar substances of colloidal nature of the cytoplasm. It is relatively abundant in vesicular but scarce in massive nuclei.

2. MULTIPLE AND DIMORPHIC NUCLEI.

While a single nucleus is characteristic of the vast majority of Protozoa, multiple nuclei are not uncommon and may be found in every group. In some forms, as in many Mycetozoa, the multinucleate condition may be due, not only to repeated nuclear divisions, but to the plastogamic union of originally independent cells, the aggregate being called a plasmodium. In other cases, as in Foraminifera, Radiolaria and Myxosporidia, the multiple nuclei are due to the incomplete division of the cell body after the nuclei have divided; or no attempt at all is made by the cell body to divide. Analogous multinucleate stages are frequently found during certain phases of the life history of many types such as the antecedent stages of sporulation and gamete formation in Rhizopoda and Sporozoa. In still other, and in the typical cases, multiple nuclei are present throughout the entire vegetative life, the number ranging from two to several hundred (*e. g.*, *Actinosphærium*). Characteristic and familiar examples of binucleate cells amongst rhizopods are *Arcella vulgaris*, *Pelomyxa binucleata*, etc.; amongst flagellates, *Giardia intestinalis* and other species of the same genus.

Multiple nuclei are found in *Pelomyxa palustris*, *Actinosphærium eichhornii*, Calonymphidæ and in the majority of Infusoria.

Dimorphic nuclei are examples of multiple nuclei in which a different function in the cell is associated with the different nuclei. Such function may be of a sexual nature as in the Myxosporidia where differences in size and structure indicate a differentiation which may be expressed by the terms male and female nuclei since products of two of them, one from each type, unite to form a fertilization nucleus of the young cell (sporozoite) according to the observations of Schroeder and Keysselitz. Or the function may be of a metabolic nature in one type and reproductive in the other, as in the Infusoria, where the two types show great differences in form and size. Here the nucleus having to do with metabolism makes up a large part of the volume of a cell and is usually of relatively large size, hence is called the *macronucleus*, while nuclei having to do with reproduction and fertilization are always minute and are called *micronuclei* (Fig. 30). Usually the micronucleus is closely attached to the macronucleus and, in some cases, may be embedded in its substance (*e. g.*, *Blepharisma undulans*) emerging only during phases of conjugation; or it may be partially hidden in a depression or pit in the macronucleus, or it may be entirely independent of the larger nucleus and lie freely in the cytoplasm. A typical example of dimorphic nuclei is shown by *Paramecium caudatum* (Fig. 22, p. 57).

The form assumed by macronuclei and the number in a single cell, varies within wide limits. The most generalized condition is a simple, spherical form; but ellipsoidal, rod-like, horse-shoe-shape, beaded and branched macronuclei are not uncommon. The beaded forms frequently appear like several separated nuclei but the segments are usually enclosed in a common membrane contracted at the nodal points, the entire aggregate forming a single nucleus (*Spirostomum*, *Stentor*, *Amphileptus*, *Uronychia*, etc.). In other cases, however, multiple macronuclei are formed by repeated nuclear divisions, the eight macronuclei of *Uroleptus mobilis*, for example, arising by three consecutive divisions of an original single nucleus (Fig. 1). The size of the macronucleus bears no constant relation to the size of the organism (Fig. 30).

Micronuclei do not differ much in form but vary in structure from typical vesicular to compact massive types. Their number in the cell likewise varies from 1 to as many as 80 or more (*Stentor*). They are never connected with one another, but are quite independent and distributed at intervals along the side of the macronuclei.

There is little or no evidence of the phylogenetic origin of these dimorphic nuclei which are distinctive of the Infusoria. In ontogenetic origin the macronuclei are invariably derived after conjugation from a division product of the fertilization nucleus, the latter being formed by the union of two micronuclear elements. Hence the

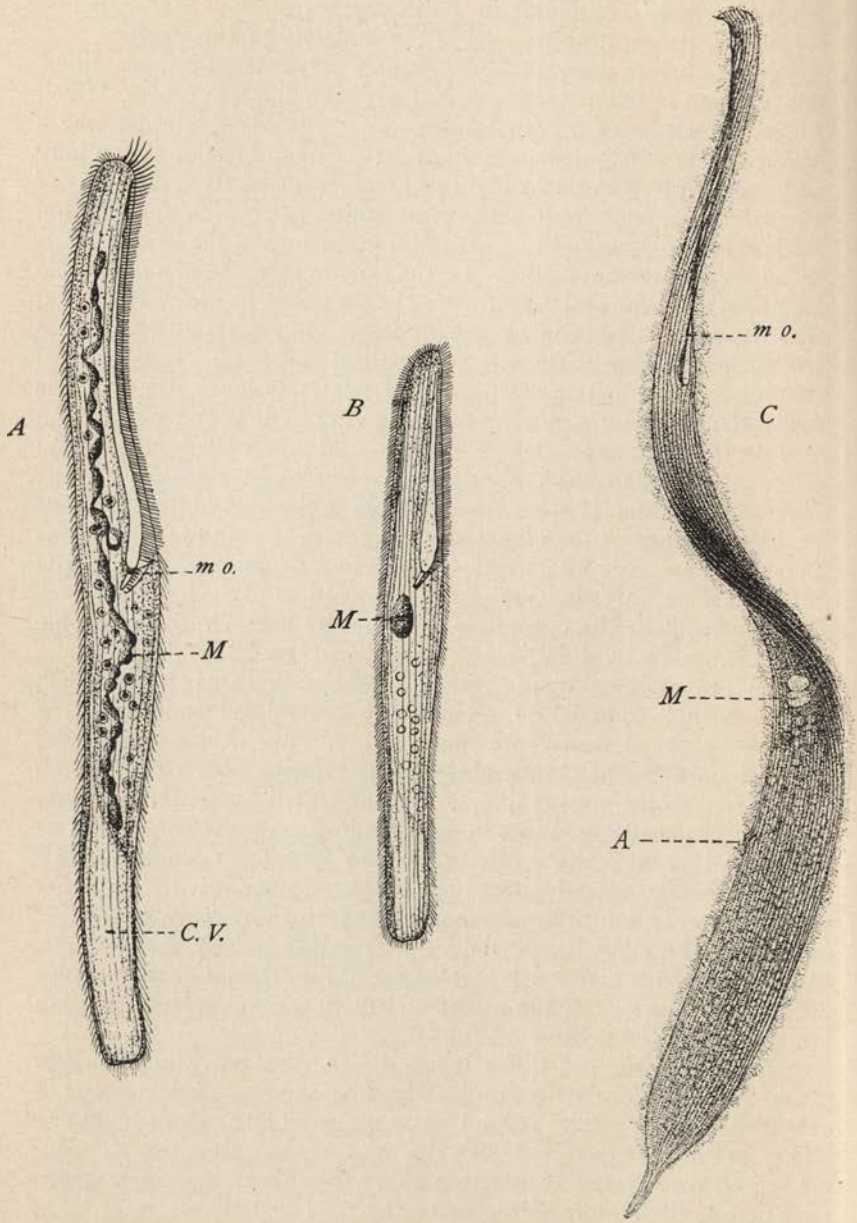

FIG. 30.—Illustrating volume relations of macronuclei and cell body. *A*, in *Spirostomum ambiguum; B*, in *Spirostomum teres;* and *C*, *Lionotus procerus;* (*a*), anal pore; (*C.V.*) contractile vacuole; *M*, macronucleus; (*mo.*) mouth. In *Lionotus* the mouth is a long slit, in *Spirostomum* a circular opening at the posterior end of the peristome. (*A* and *B* after Stein; *C*, original.)

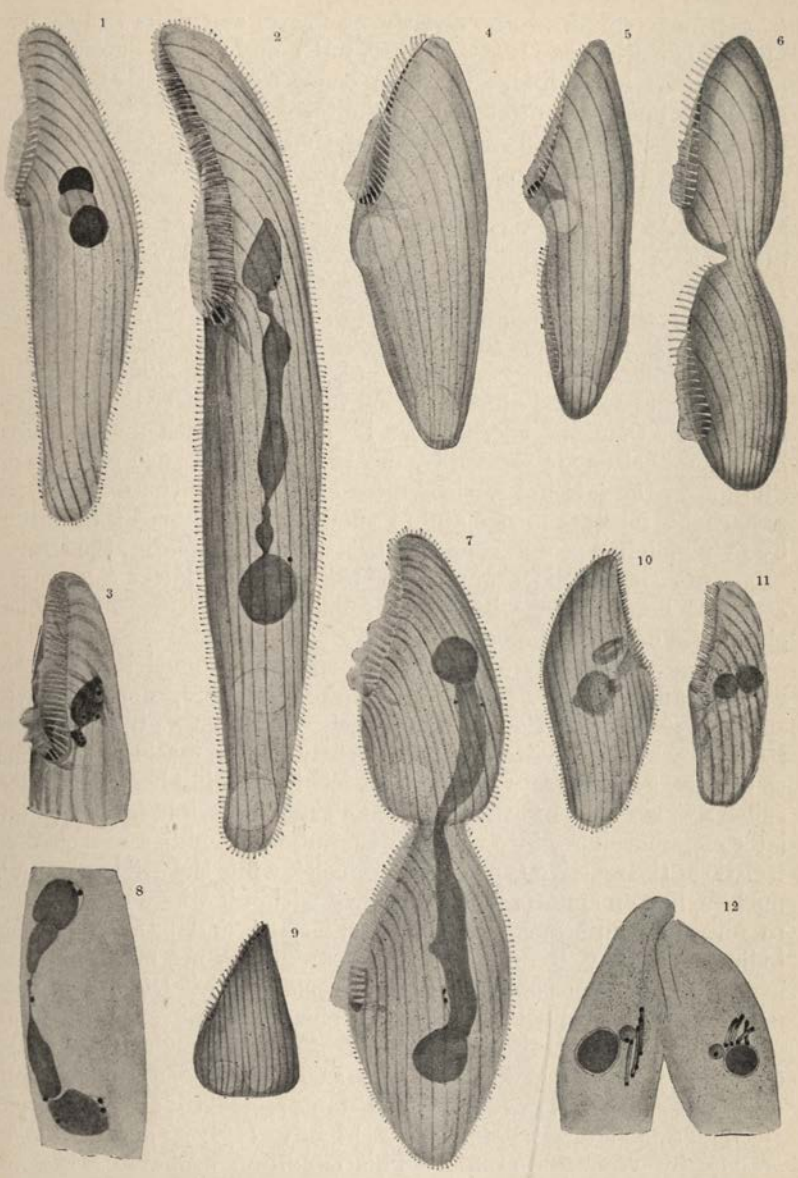

FIG. 31.—Division of *Blepharisma undulans*. Here the micronuclei are inside the nuclear membrane of the macronucleus. (1) Normal vegetative individual; (2) elongated cell and nuclear division; (3) details of oral structures and nuclei; (4 to 7) stages in cell division; (8) relations of macro- and micronuclei; (9) young cell; (10 and 11) ex-conjugants; (12) second meiotic division during conjugation. (After Calkins.)

statement is usually made that macronuclei arise from micronuclei, a statement which is not strictly accurate, since the fertilization nucleus is neither one nor the other, but merely a cell nucleus of an unorganized individual. In some cases macronuclei and micronuclei are not differentiated until the third division of the fertilization nucleus (*e g.*, in *Cryptochilum nigricans, Paramecium caudatum, Par. putrinum, Bursaria truncatella, Carchesium polypinum, Opercularia coarctata, Ophrydium versatile, Vorticella monilata, V. nebulifera*, etc.); in other cases differentiation occurs after the second divisions (*e. g.*, in *Anoplophrya branchiarum, Colpidium colpoda, Didinium nasutum, Glaucoma scintillans, Leucophrys patula, Lionotus fasciola, Paramecium aurelia, Par. bursaria, Blepharisma undulans, Spirostomum teres, Euplotes patella* and *charon, Onychodromus grandis, Stylonychia pustulata, Uroleptus mobilis*, etc.); and in still other cases the differentiation takes place after the first division (*e. g., Chilodon uncinatus*). In all cases both macronucleus and micronucleus are formed by metamorphosis of such products of division of the original nucleus after conjugation, the former by a remarkable increase in size and in quantity of chromatin, the latter by reduction in size and concentration of the chromatin; the former becomes a metabolic organoid of the cell, the latter a germinal organoid homologous with the chromidiosomes representing idiochromatin of the rhizopods.

A suggestive history of differentiation of macronuclei and micronuclei is afforded by *Blepharisma undulans* Here, after two divisions of the fertilization nucleus, each of the four products gives rise not by a third division, but apparently by chromatin transfusion, to a large, homogeneous and at first feebly-staining body originally called a "placenta" by Bütschli. The exuded, peripheral chromatin later metamorphoses into the large granular chromidiosomes characteristic of the massive type of macronuclei, while the original central nucleus, like an endosome, is contained within it where it condenses to form the minute micronucleus (Figs. 31 and 32). A similar hiding place may account for the apparent absence of micronuclei in forms like *Actinobolus radians, Lacrymaria olor, Didinium nasutum*, etc. Amicronucleate races of ciliates, however, have been cultivated by several observers: *Didinium* by Patten (1921); *Oxytricha fallax* by Woodruff (1921); *O. hymenostoma* by Dawson (1919); *Paramecium caudatum* by Landis (1920) and Woodruff (1921); *Spathidium spathula* by Moody (1912); and *Urostyla grandis* by Woodruff (1921). This condition probably arises by faulty reorganization after conjugation, but is also characteristic of old-age ciliates.

Endosomes are comparatively rare in these dimorphic nuclei but may be present in the form of (?) plastin nucleoli (macronucleus of *Epistylis plicatilis* according to Schröder), or as endobasal bodies in

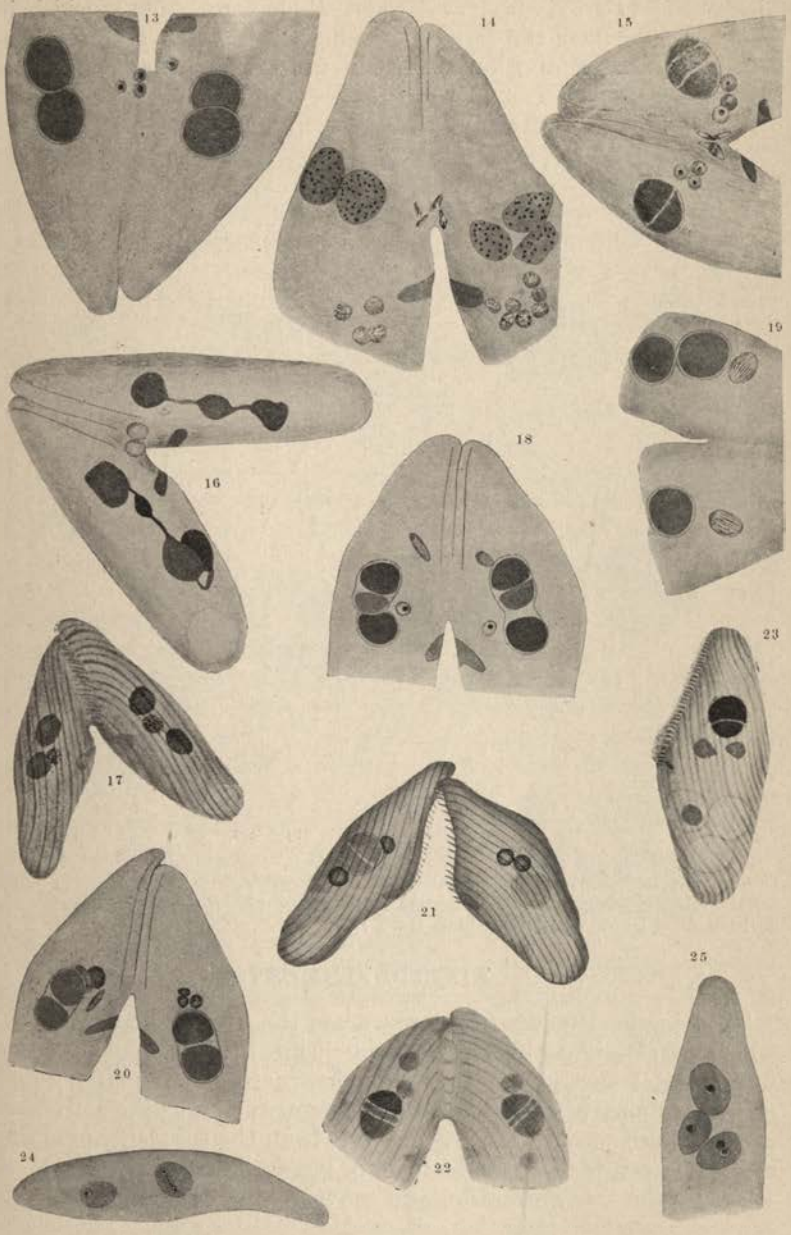

FIG. 32.—Conjugation of *Blepharisma undulans* (continuation of Fig. 31). (13 to 16) interchange and fusion of gametic nuclei; (18 to 20) first division of the amphinucleus; (24, 25) origin of new macronucleus from progeny of the amphinucleus, the micronuclei remaining in the macronuclei. (After Calkins.)

micronuclei (*Paramecium caudatum*, *P. bursaria*, etc.), which in some cases assumes the form of a centriole, forming a typical centrodesmus during division (*e. g.*, in first maturation spindles of *Uroleptus mobilis*).

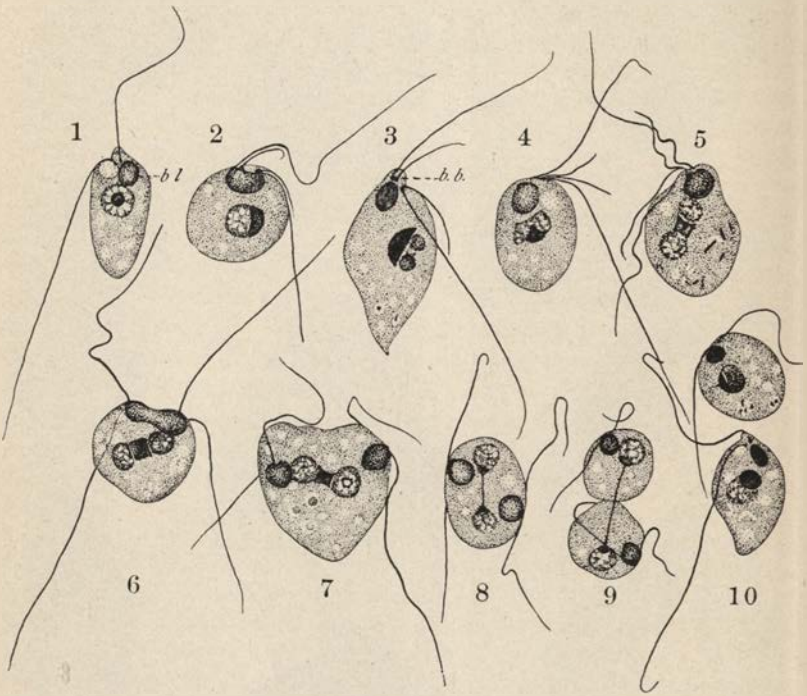

FIG. 33.—*Bodo ovatus* Stein (*edax*, Belar). (1) Vegetative individual with two flagella; blepharoplast (*bl*) and nucleus with endosome. (2 to 6) Division of the basal bodies, blepharoplast and nucleus; (7 to 10) completion of nuclear division and division of cell body. (After Belar, from Doflein.)

3. KINETIC ELEMENTS.

The kinetic elements of Protozoa are those structures of the cell which are closely connected with the visible expression of the transformation of energy resulting from destructive metabolism. Such expression may be in the form of movement due to the activity of specific motile organs formed as a rule from the substance of kinetic elements, or it may be in the form of intracellular activities as indicated by the transformation and movements of internal attraction centers, center of radiation, of nuclear division, etc. The kinetic elements are justly regarded by many observers as the most elusive and perplexing, but at the same time the most fascinating of all the organoids of Protozoa.

Kinetic elements appear in Protozoa in a multitude of structures, sometimes intranuclear, sometimes cytoplasmic, and often both inside and outside the nucleus. Whether or not they are permanent organoids of the cell is subject to the same arguments pro and con which have been raised for and against the permanency of the centrosome in Metazoa. There is strong evidence, as the following pages will show, that not only are many types of cytoplasmic kinetic elements derived from the nucleus, but also that chromatin and intranuclear endobasal bodies are closely related, while some types that are confined to the cytoplasm are composed in part, or entirely, of a substance which closely resembles chromatin (parabasal bodies). Little is known of the chemical composition of the latter, but they stain intensely with some of the nuclear dyes and divide by simple constriction at periods of cell division.

The kinetic elements vary in complexity from simple homogeneous spheres and granules to extremely complicated systems of masses and fibers, to which, in some cases, a sensory and conductile function has been attributed in addition to the primary functions associated with movements. To these more complex types Kofoid applies the name "neuromotor" systems, a suggestive term first used by Sharp (1914) in describing the characteristic structures and supposed functions of the kinetic elements in *Diplodinium ecaudatum*. In general, they appear to be more highly differentiated in parasitic than in free-living types of Protozoa where, as Kofoid (1916) points out, the denser media in which they live and have to move, such as blood, mucus, intestinal contents, etc., require more powerful motile organs and better developed kinetic centers than do water-dwelling forms. On the other hand, free-living forms have not been so extensively and carefully studied as the usually more minute parasitic types and the field of investigation opened by the observations of Yocom (1918) and the experiments of Taylor (1920) on *Euplotes patella* indicate that free-living ciliates are not far behind in this line of differentiation (see infra p. 109).

In many cases it is impossible to tell from observations on ordinary vegetative individuals, whether a given structure belongs to the kinetic elements or to some other group of the many types of protoplasmic granules. This is particularly true of the intranuclear forms where incomplete extraction of a stain may give the appearance of a granule in some chromatin or plastin mass. In such cases the identity of the structure can be determined only by its history during nuclear division. Cytoplasmic forms can be more easily detected by reason of their relation to motile organs or to more or less complex fibrillar structures.

(*a*) **Intranuclear Kinetic Elements (Endobasal Bodies).**—Endobasal bodies in nuclei of different Protozoa are highly variable and no general description is possible. In some cases they stain intensely

with nuclear dyes, especially with iron hematoxylin (Euglenida); in other cases they stain feebly or not at all with the same dyes that color the chromatin (*e. g., Chilodon*). In some cases they are large and appear homogeneous throughout; in other cases there is a definite, deeply-staining central granule embedded in a more faintly staining matrix, or such a granule may be present without the accompanying matrix; or, finally, there is no evidence at all of kinetic elements in resting nuclei, but collections of homogeneous substance are present at the poles of the nucleus during division (pole plates).

1. *Large Homogeneous Endobasal Bodies.*— In this type the endobasal body is conspicuous by its large size and homogeneous structure. It was first described by Keuten (1895) in *Euglena viridis* and was early recognized as a kinetic element connected with nuclear division as attested by the names intranuclear centrosome, division center, etc., applied to it, while nuclei containing it were included by Boveri in his "centronucleus" type. In *Euglena viridis* and euglenoids generally, this endobasal body according to earlier descriptions of Keuten, Tschenzoff (1916) and others, is the most conspicuous structure of the nucleus, where, in the resting nucleus it appears as a spherical or elongated ellipsoidal body with chromatin granules of limited number suspended between it and the nuclear membrane (Fig. 25, p. 62). It divides prior to division of the chromatin, first elongating with a concentration of its material at the poles (*B, C*). The elongation continues until a thin fibril, called a centrodesmose, alone connects the two halves (*C, D*). The centrodesmose ultimately breaks and its substance is absorbed by the two daughter elements (*D*). According to more recent observations of Baker and of Hall (1923), however, there is an extranuclear blepharoplast which divides with connecting paradesmose, the daughter blepharoplasts as centrioles forming the poles of the spindle. (See also *Oxyrrhis*, Fig. 43.) In the rhizopod *Chlamydophrys stercorea*, as well as in the flagellate *Bodo ovatus*, the endobasal body which is quite similar to that of *Euglena*, divides subsequently to division of the chromatin (Schaudinn, Belar, Fig. 33), while in *Amœba crystalligera* (Schaudinn) there is no centrodesmose formed during division, a condition not uncommon in the rhizopods (*e. g., Arcella vulgaris* according to Swarczewsky; *Vahlkamfia limax* (Fig. 26), and many species of *Endamœba*). Not only is this simple type of endobasal body found in rhizopods and flagellates, but also in some cases in the more complex ciliates, where, in *Chilodon cucullus*, for example, the macronucleus contains a definite endosome which behaves exactly like that of *Euglena* (Fig. 34). It is highly probable that in all of these cases the endobasal body is embedded in a core of plastin.

2. *Endobasal Bodies with Centrioles.*— Centrioles are kinetic elements in the form of minute granules, which in Metazoa and in

some types of Protozoa, form the focal points of the mitotic spindle. In many Protozoa minute granules may be embedded in a matrix

FIG. 34.—*Chilodon* sp. Macronucleus with endosome and endobasal body (end) (*mo*) Mouth surrounded by pharyngeal basket. (Original.)

of chromatin or plastin, or in a combination of both. These in some cases form the poles of typical spindles, but in the majority of cases, apart from the polar granules and the connecting centrodesmose, there is little evidence of a typical spindle.

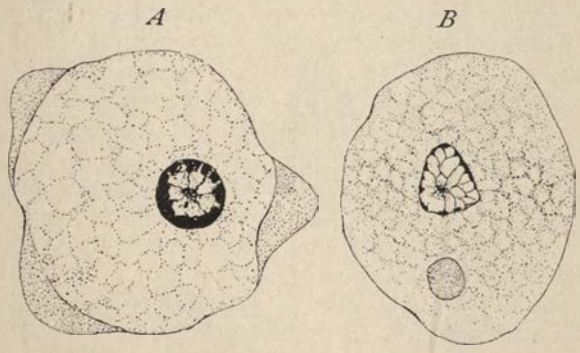

FIG. 35.—*Endamœba dysenteriæ* (Councilman and Lafleur). Two stages in the metamorphosis of endosome and endobasal body. (After Hartmann.)

In some cases this type of endosome undergoes changes in appearance which Hartmann (1911) and his followers have interpreted as

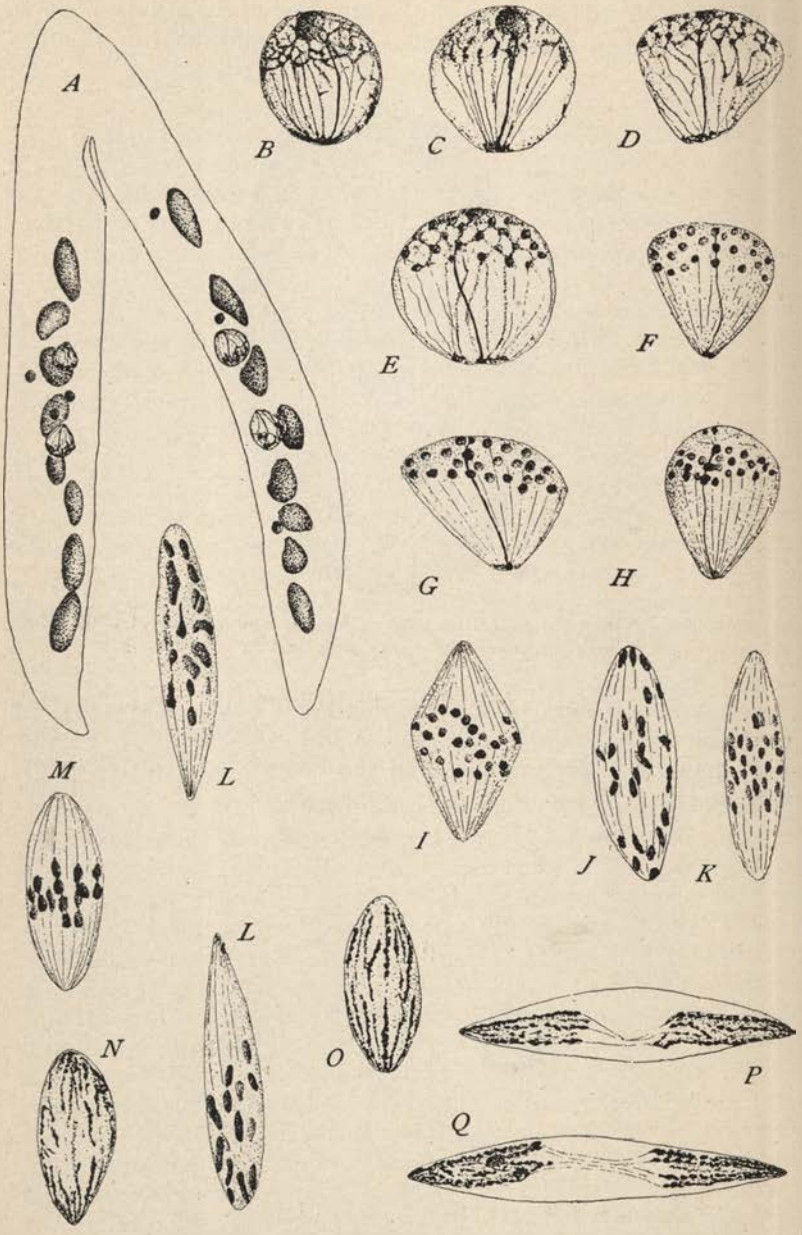

FIG. 36.—*Uroleptus mobilis* Eng. First and second meiotic divisions during conjugation. (*A*) Two conjugating individuals; (*B* to *G*) formation of the first spindle pole by division of the endobasal body (with centrodesmose); (*H* to *M*) first meiotic nuclear division; (*N* to *Q*) second meiotic division. (After Calkins.)

periodic or cyclical in nature. Such variations have to do with the concentration of the chromatin substance about the endobasal body or centriole, being massive and dense in certain phases and distributed in others. In *Endamœba dysenteriæ* the centriole in the latter phase is distinct and definite but in the former phase it is hidden by the dense chromatin (Fig. 35). From such conditions Hartmann infers that all massive types contain hidden centrioles, a conception applied by Naegler to all of the smaller amœbæ and endamœbæ, but is limited to comparatively few types according to Gläser.

Typical endobasal bodies in the form of centrioles are contained in the first maturation nuclei of *Uroleptus mobilis*. Here each massive micronucleus fragments into chromatin granules which remain in a dense reticulum at one pole of the enlarging nucleus until the chromosomes are formed. A centriole, hidden in this mass, divides and one-half traverses the nucleus to form the first pole of the maturation spindle but remains connected by a centrodesmose with the other centriole which, in turn, forms the other pole of the spindle (Fig. 36, *b*, *g*). Similar centrioles are found in widely separated groups of Protozoa. In *Coccidium schubergi*, according to Schaudinn (1900), the endobasal body divides with a long connecting centrodesmose. Here, however, part of the material of the centrodesmose collects into two granules with a more densely stained connecting thread, thus producing a structure which Doflein interprets as analogous to the mid-body (Zwischenkorper) of Metazoa and plant cells. In *Polytomella agilis* as described by Aragao, in some trypanosomes, and in many minute amœbæ, centrioles showing a similar history are of frequent occurrence (see Chatton, Nägler, Glaser, *et al.*). In *Amœba diplomitotica*, Aragao (1904) has also described two types of endobasal bodies in the same species. One resembles the homogeneous endosome of *Euglena viridis* in having no centriole (Keuten), while the other type consists of a substance similar to that of the first type within which a centriole is embedded the latter forming a typical centrodesmose during division. It is probable, however, that this supposed difference is only a matter of technic. The fate of the centrioles after division differs in different cases. In some *e. g.*, *Bodo lacertæ*, Belar, 1921, Figs. 37, 38), they come from the nucleus and reënter the daughter nuclei;* in others they arise from basal bodies and become basal bodies of the flagella after division (*e. g.*, *Chilomastix aulostomi*, Belar, 1921; *Parapolyptoma*, Jameson, *Spongomonas*, Hartmann, etc.).

While the embedding matrix in most of the above cases is similar to chromatin in its reaction, and forms an important part of the

* See, however, the earlier contradictory accounts of Prowazek (1904), Alexeieff (1914), and Kuczynski (1918).

endobasal body, there are other types (*e. g., Myxobolus pfeifferi*, one of the Myxosporidia) in which the centriole emerges from an enveloping plastin-like matrix, which, like a nucleolus, then degenerates and disappears.

An interesting variation of this type of endobasal bodies is illustrated by *Amœba vespertilio* as described by Doflein. Here the endosome is composed of chromatin, plastin and kinetic elements and all parts of the spindle are made up solely from these endosomal substances, while the outer nucleus appears to be passively divided

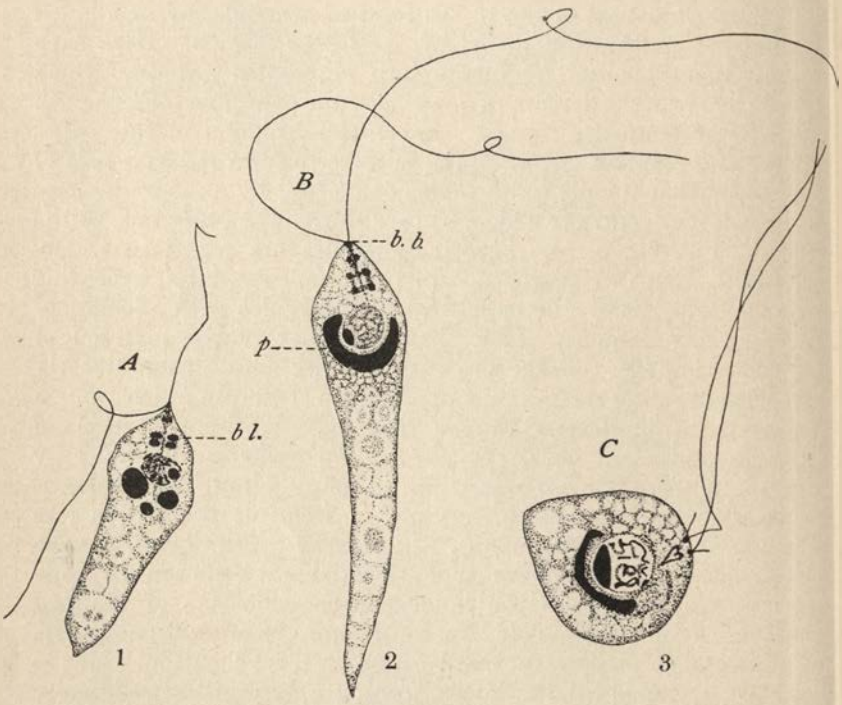

FIG. 37.—*Bodo lacertæ* Grassi. Early stages of division of the basal bodies, (*bb*); blepharoplast ring (*bl*); nucleus and parabasal body (*p*). (After Belar.)

(Fig. 39). In contrast with this may be cited the observation of Enriques (1913) who found complete spindles without trace of chromatin.

Centrioles, finally, may be present without other covering or enveloping substances as in the case of *Paramœba chætognatha* (according to Janicki, 1912), or in *Centropyxis aculeata* according to Schaudinn, 1903). In the former a centrodesmose is formed during division stages; in the latter no centrodesmose occurs but the centrioles at the poles of the mitotic spindle, as in a metazoön

astrosphere, form attraction centers, not only for the spindle fibers, but also for astral rays extending into the cytoplasm.

3. *Nuclei with Pole Plates and Without Endobasal Bodies.*— This type of nucleus is characterized by the entire absence of endobasal bodies. A hyaline mass, which stains with difficulty, may, however, be present at the spindle poles during nuclear division, but in

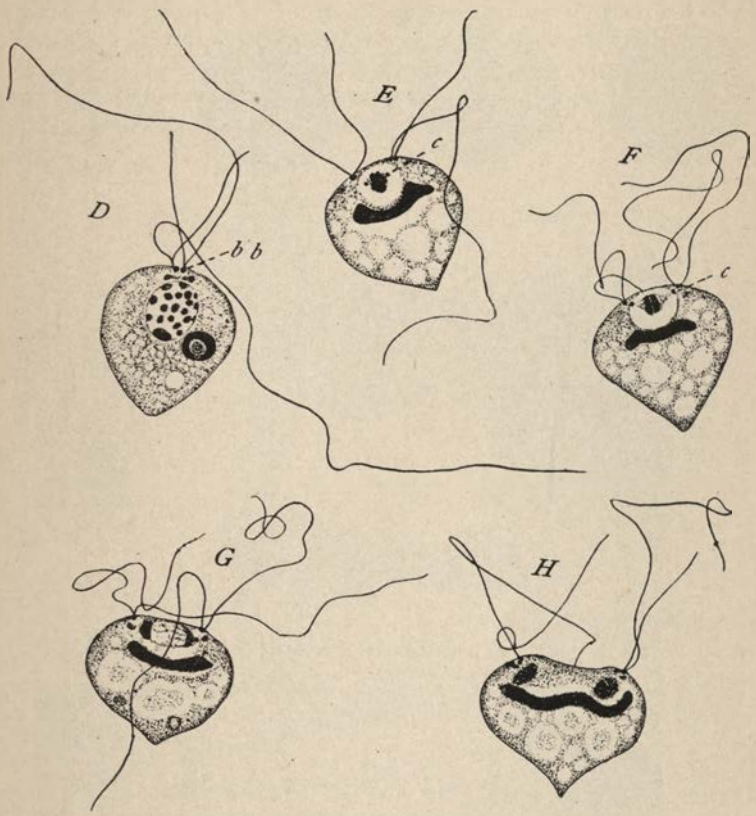

FIG. 38.—*Bodo lacertæ* Grassi; division stages continued. (*E*) Origin of centriole in the nucleus, and their retention in the daughter nuclei, (*F* to *G*); (*bb*) basal bodies (*c*) centriole. (After Belar.)

many cases it cannot be detected in the resting nucleus. During division it occurs in characteristic forms known as pole plates.

In the micronuclei of *Paramecium caudatum* such a mass forms a hyaline cap at one pole of the otherwise chromatin-filled resting nucleus. Observations are entirely lacking in regard to division of this mass during reproduction, but similar aggregates of non-staining substance are present at the distal ends of the daughter

nuclei during stages of division (Fig. 40). Similar pole plates appear as broad, flat, and hyaline ends of the spindles of *Actinosphærium eichhornii* according to Hertwig (1898), in the spindle of *Trichosphærium sieboldi* according to Schaudinn (1899), and in the macronucleus of *Spirochona gemmipara* (Hertwig). In this group, also, we would include the peculiar hyaline globular bodies at the poles

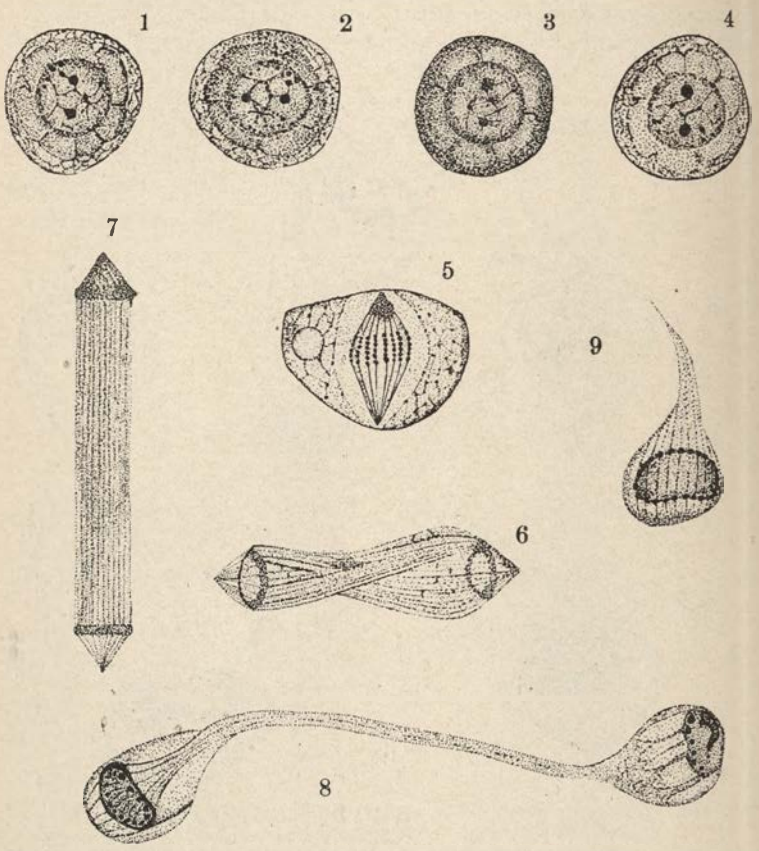

FIG. 39.—*Amœba vespertilio* Dof. Origin of the spindle within the nucleus (1, 2), nuclear division (5, 6, 7), and reconstruction of nuclei after division (3, 4, 8, 9). (After Doflein.)

of the nuclear spindles of *Euglypha alveolata* as described by Schewiakoff (1888).

It is quite possible, although direct evidence is lacking, that none of these peculiar pole plate structures belongs to the group of kinetic elements. Indirect evidence favoring this possibility is furnished by the entire absence of observations on the division of a

definite body, the substance of which forms the pole plates. Hertwig (1898) and Doflein (1916) assume that they are formed from the linin substance of the nucleus. On this assumption the pole plates might be interpreted as hyaline aggregates of the linin reticulum of the nucleus, indeed, the hyaline and homogeneous appearance of the pole plates is suggestive of amœba ectoplasm. With our present knowledge I am inclined to agree with this interpretation of pole plates and to regard *Paramecium caudatum*, with other species of this genus, *Actinosphærium eichhornii* and the other forms mentioned above, as containing no intranuclear kinetic elements. To such a group we would also assign forms like *Aulocantha scolymantha* and *Chilomonas paramecium*, in which according to observations of

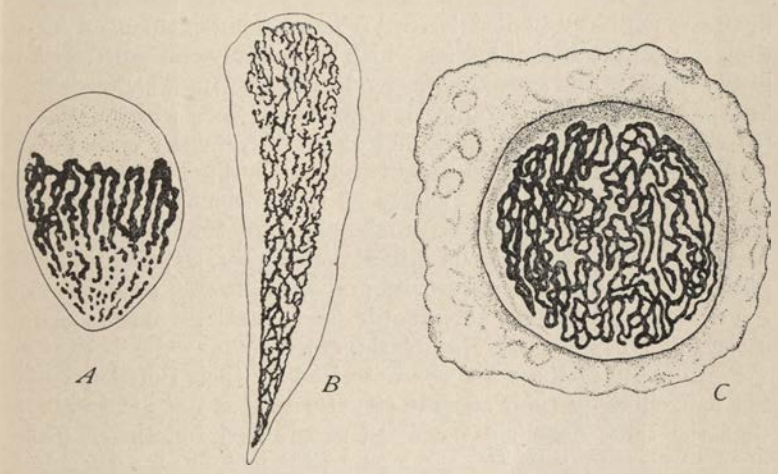

FIG. 40.—Micronucleus of *Paramecium caudatum* in the prophases of the first meiotic division. *A*, Early stage in the formation of chromosomes; *B*, elongation of the nucleus prior to crescent formation; *C*, metaphase of the first division. Dehorne describes the entire chromatin aggregate as forming one highly convoluted chromosome. (After Dehorne.)

Borgert (1909) and Alexeieff (1911), not only intranuclear kinetic elements but pole plates as well are entirely absent.

(*b*) **Extranuclear (Cytoplasmic) Kinetic Elements.**—It is in the cytoplasm that kinetic elements are most highly differentiated, and the often perplexing structures which appear in different types of Protozoa have led to much confusion in terminology. Any attempt, therefore, to present a clear picture of the diverse elements and to distinguish one type from another, inevitably leads to contradictions in connection with interpretations of one or another observer. The facts may be marshalled, however, into a fairly logical and consistent series indicating an increasing complexity in the organization of the cell. Such a series is presented in the following pages with

the understanding that it involves no claim of finality, nor does it indicate phylogenetic relationships.

The kinetic structures most frequently found in the cytoplasm of Protozoa are relatively simple, the more complex types which have been revealed being found in comparatively few cases. In considering Protozoa as a group, therefore, too much weight should not be attributed to these more complicated forms. For purely descriptive purposes they may be considered in the following order: (1) Kinetic elements, which are morphologically and functionally equivalent to intranuclear centrioles forming parts of endobasal bodies and usually derived from them; (2) blepharoplasts equivalent to basal bodies, or independent of basal bodies, which lie at or near the bases of motile organoids and give rise to the kinetic structures in them; (3) basal bodies derived from and independent of blepharoplasts; (4) parabasal bodies which are closely connected with the blepharoplasts and probably derived from them; (5) centrodesmoses and paradesmoses, or connecting fibrils between kinetic elements; (6) rhizoplasts, or fibrils originating as outgrowths from the substance of specific kinetic elements and connecting two such elements or ending blindly in the vicinity of the nucleus; (7) astrospheres and centrosomes, similar to analogous structures in the cells of Metazoa; (8) miscellaneous kinetic elements such as centroblepharoplasts, axostyles, parastyles and the neuromotor apparatus of flagellates, "motorium," conductile fibrils, and myonemes of Infusoria, myophrisks of the Radiolaria, etc.

Since many of these are characterized by their functional activities as well as by their specific structures, it is not illogical to find that the same organoid performs generalized functions. Thus a blepharoplast may be the same as a centriole, or as a basal body; rhizoplasts may arise as a broken centrodesmose or paradesmose; a myoneme as a conductile element, etc. The complexities of organization arise from the simultaneous presence of many of these different kinetic elements in the cell where they may form a coördinating system of organoids which Sharp and Kofoid have aptly designated the *neuromotor system.*

1. *Blepharoplast, Basal Body and Centriole.*—In many of the comparatively simple Protozoa which have no specialized motile organoids, the cytoplasm apparently lacks all traces of specific kinetic elements. Thus in the entire group of Sporozoa, in the simpler Gymnamœbida and in testate forms of rhizopods, kinetic elements, if present at all, are in the form of endobasal bodies within the nucleus or as centrosomes close to it. Arndt (1924) however, has recently described a centrosome, with centriole, which divides and forms the poles of the mitotic figure in *Hartmannella* (*Pseudochlamys?*) *klitzkei*, a testate rhizopod (Fig. 41). In some of the relatively simple rhizopods, however, especially those belonging to

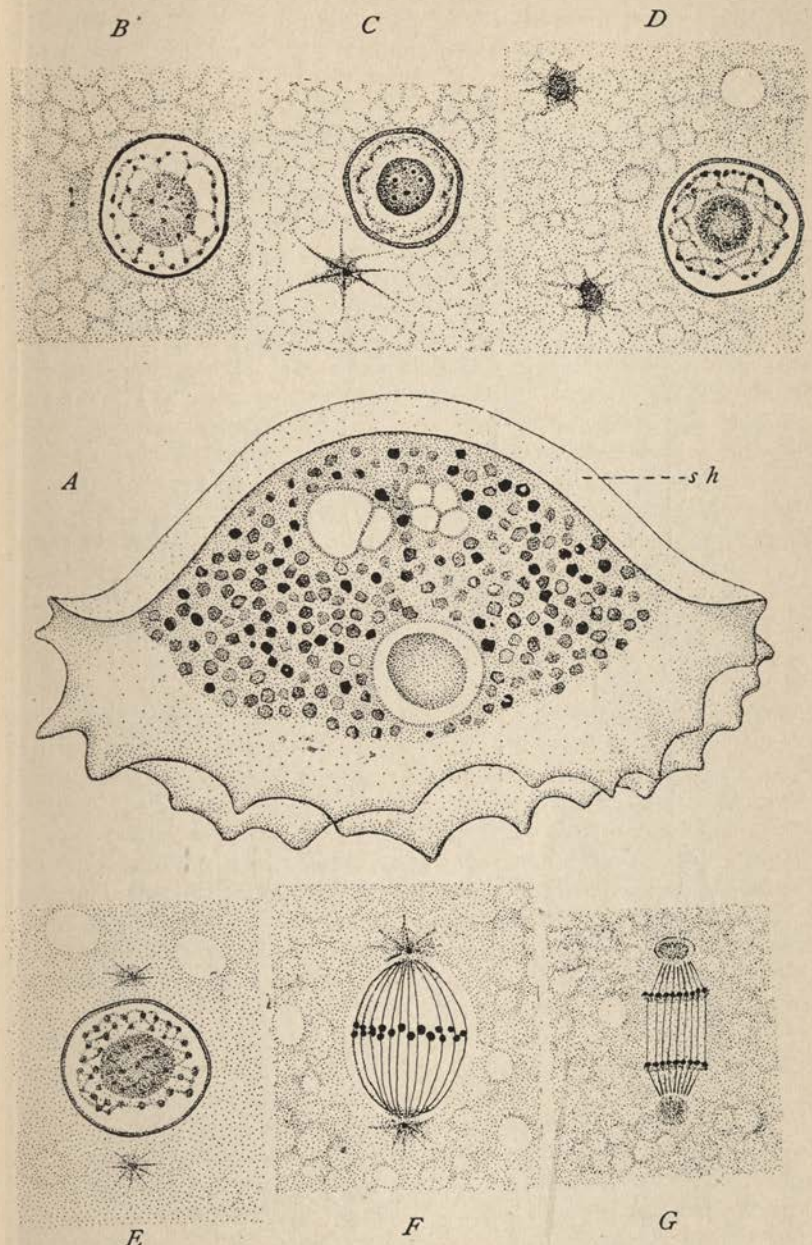

FIG. 41.—*Hartmannella* (*Pseudochlamys ?*) *klitzkei* Arndt. Centrosome and centriole in a testate rhizopod. *A*, Animal with watch-glass-like shell; *B* to *F*, origin of the centrosome in the cytoplasm, its division, and position on the spindle; *G*, anaphase stage of nuclear division. (After Arndt.)

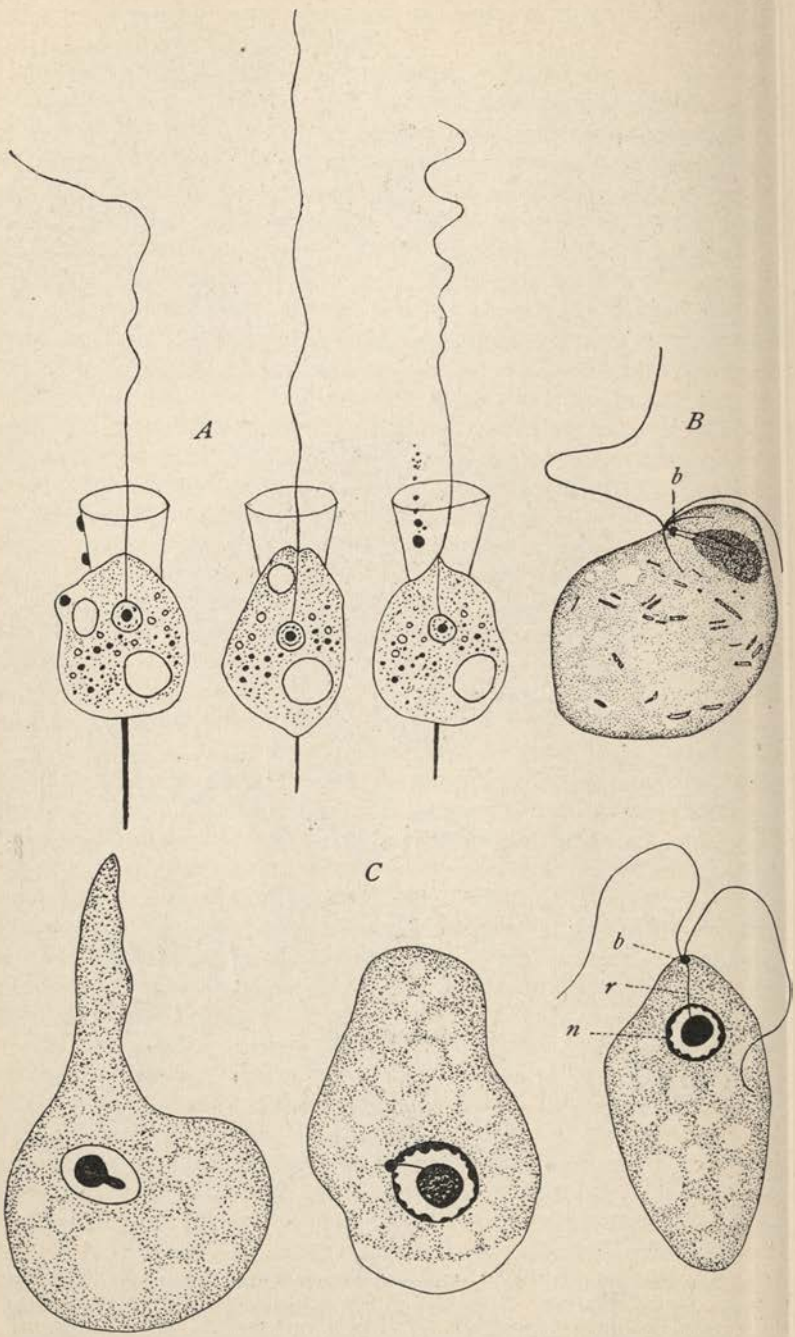

FIG. 42.—Flagellum insertion. *A*, *Codosiga botrytis*, with flagellum arising from the nucleus. *B*, *Nägleria bistädialis* Pusch. with blepharoplast connected by rhizoplasts with the nucleus, and with independent basal bodies. *C*, *Nägleria gruberi* and origin of the blepharoplast from the endosome in the nucleus; (*b*) blepharoplast; (*n*) nucleus; (*r*) rhizoplast. (*A* and *B* from Doflein, *C* from Wilson.)

the family which Doflein has called the Bistadiidæ, from the fact that two distinct phases—an amœboid and a flagellate phase— are interchangeable, we find organisms which throw light on the origin of cytoplasmic kinetic elements. Such dimorphic types of rhizopods have been repeatedly observed since Dujardin first called attention to them, but details concerning the origin of kinetic elements and the flagellum have been made out only through use of modern cytological methods.

In some Protozoa, *e. g.*, *Codosiga botrytis*, the kinetic elements of the flagellum grow directly out of an endobasal body in the nucleus, indicating their origin from an intranuclear kinetic element (Fig. 42, *A*), in other simple forms the flagellum arises from a kinetic element situated in the cytoplasm but connected with the intranuclear kinetic element by a rhizoplast at some stage (Fig. 42, *B*). In *Polytoma uvella* according to Geza Entz (1918), the relations between intranuclear and cytoplasmic kinetic elements varies with the age of the cell. The usual condition in adult cells is two basal bodies, one at the base of each flagellum, and neither of them is connected by a rhizoplast with the nucleus. In young individuals, however, the original single blepharoplast (= basal body) is connected by a rhizoplast with an intranuclear endobasal body, or a larger rhizoplast from the blepharoplast may break up into a calyx of fibrils which enter the nucleus at different points. The inference might be drawn in all such cases that the cytoplasmic body represents one of the daughter halves formed by division of the nuclear endobasal body, while the connecting fibril represents the rhizoplast formed during such division. These stages are well illustrated by the dimorphic forms of rhizopods during the transition from the amœboid to the flagellated phase. Thus Whitmore describes a cytoplasmic kinetic element functioning as a basal body which is connected by a fibril with the nucleus and which lies at the base of the flagella in *Trimastigamœba philippinensis*, and Puschkarew described a similar condition in *Nägleria punctata* (Fig. 42). The most complete observations, however, were made by Charlie Wilson in connection with the transition from amœboid to flagellated stage in a closely-related form, *Nägleria gruberi*, one of the soil amœbæ. She describes the nucleus of this organism as containing a typical endosome within which an endobasal body is embedded. At the period of flagellation this endobasal body divides and one daughter element migrates through the substance of the endosome and through the nucleus to the cytoplasm, retaining its connection throughout with the intranuclear kinetic element (Fig. 42, *C*). In the cytoplasm it becomes a basal body which gives rise to the kinetic elements of the flagella. In these cases the extruded kinetic element combines the functional characteristics of a blepharoplast and a basal body or group of basal bodies. In this dual capacity it

may be regarded as a blepharoplast—basal body. In *Nägleria bistädialis* according to Puschkarew it divides, one part remaining

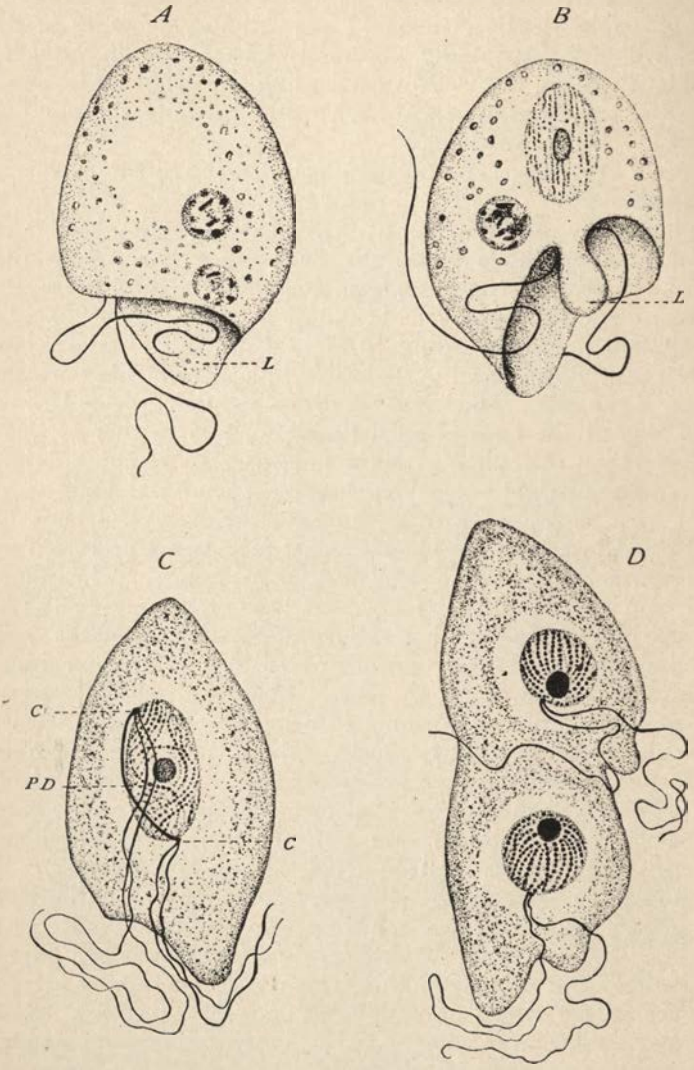

FIG. 43.—*Oxyrrhis marina* Duj. *A*, *B*, front and side views of individual with lobe (L); *C*, division of centriole (*c*); connecting strand or paradesmose (*pd*); and chromosome formation in the nucleus; *D*, beginning of cell division. (After Hall.)

as a blepharoplast, the other becoming a basal body; the two parts, however, are connected by a rhizoplast and rhizoplasts connect the blepharoplast with the endobasal body (Fig. 42, *B*).

A modification of this mode of origin of the cytoplasmic kinetic element is shown by *Parapolytoma saturna* according to Jameson (1914) and by *Oxyrrhis marina* (Hall, 1925). In these cases the old flagella and their basal bodies are said to degenerate and disappear prior to, or during, nuclear division. An intranuclear endobasal body which is concealed within an endosome during vegetative

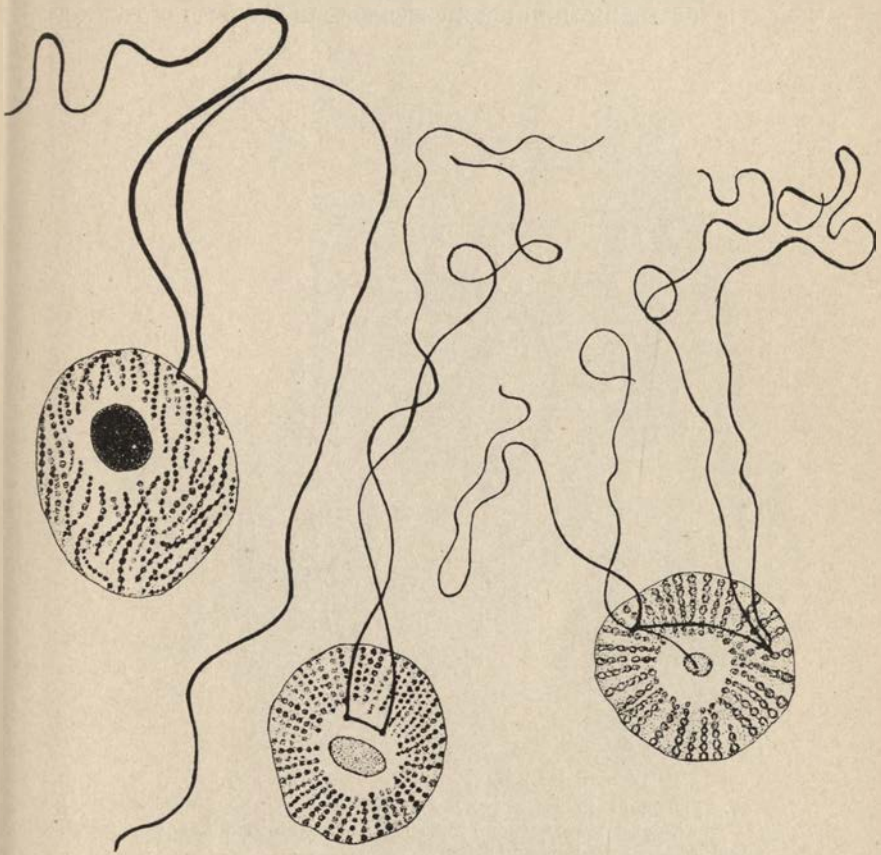

FIG. 44.—*Oxyrrhis marina;* details of nucleus, centriole, and paradesmose. (After Hall.)

life, first divides, its daughter halves forming the poles of the nuclear spindle (Figs. 43 and 44). Flagella may either grow out from the substance of the kinetic elements while the latter are still at the spindle poles, or from the endobasal body after the daughter nuclei are established.

In *Bodo lacertæ* according to Belar the centrioles after division are taken into the daughter nuclei. Here the kinetic elements,

although originating from an endobasal body, are different in function from those described in the preceding paragraph. Forming the poles of the mitotic spindle they are correctly described as centrioles, but apparently they again become endobasal bodies (Fig. 38, p. 81).

While the flagella appear to emerge directly from the nucleus in some cases, *e. g.*, in *Mastigamœba invertens* according to Prowazek, or *Codosiga botrytis* according to Doflein, in many cases they take their origin actually from kinetic elements in the form of centrioles

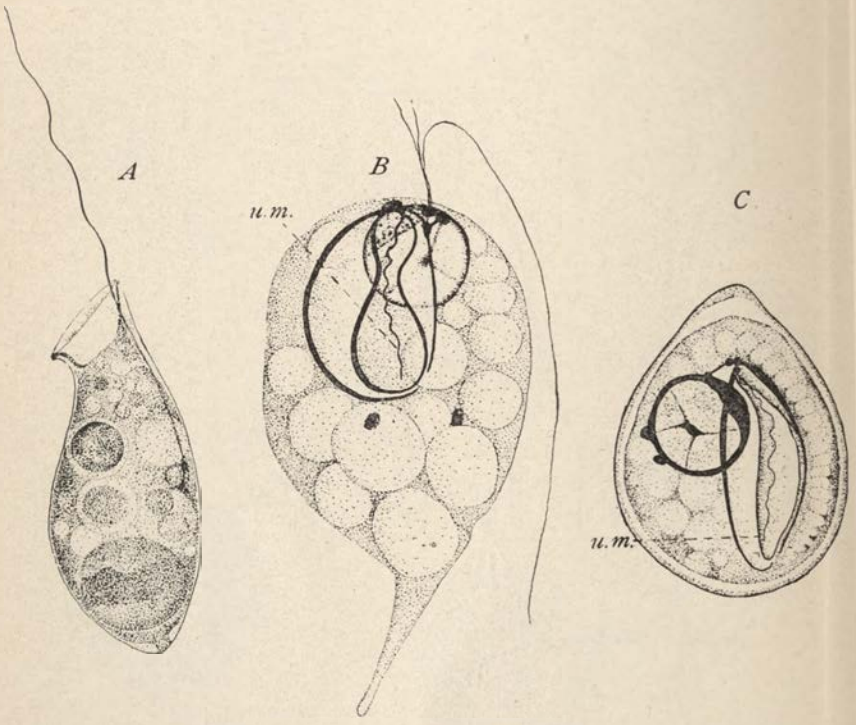

FIG. 45.—Flagellum insertion. *A*, *Phialonema cyclostomum; B*, *Chilomastix mesnili; C*, the same, encysted. (*u.m.*) Margin of undulating membrane in cytostome. (*A*, Original; *B*, *C* after Kofoid and Swezy.)

which lie on the outside of the nuclear membranes, as in *Mastigina setosa*, *Phialonema cyclostoma*, *Cercomonas longicauda*, *Oicomonas termo*, or *Chilomastix gallinarum* (Fig. 45). In such cases, illustrated by *Chilomastix aulostomi* according to Belar (1921), centrioles, become the basal bodies, and the latter become centrioles. In such cases the basal bodies are unquestionably blepharoplasts.

In other cases the blepharoplast does not remain connected with the nucleus by any fibrillar process, but as an entirely separated

and independent kinetic element gives rise to the flagella at or near the anterior end of the cell (*Leptomonas jaculum*, *Scytomonas subtilis*, Dobell (1908), *Scytomonas pusilla*, Schüssler (1918), or *Herpetomonas gerridis* (Fig. 96). In *Chilomastix mesnili* Kofoid and Swezy (1920) describe three blepharoplasts, one of which gives rise to two flagella, another gives rise to one flagellum and the parastyle, the third to the parabasal, peristomial fibril, and the cytostomal

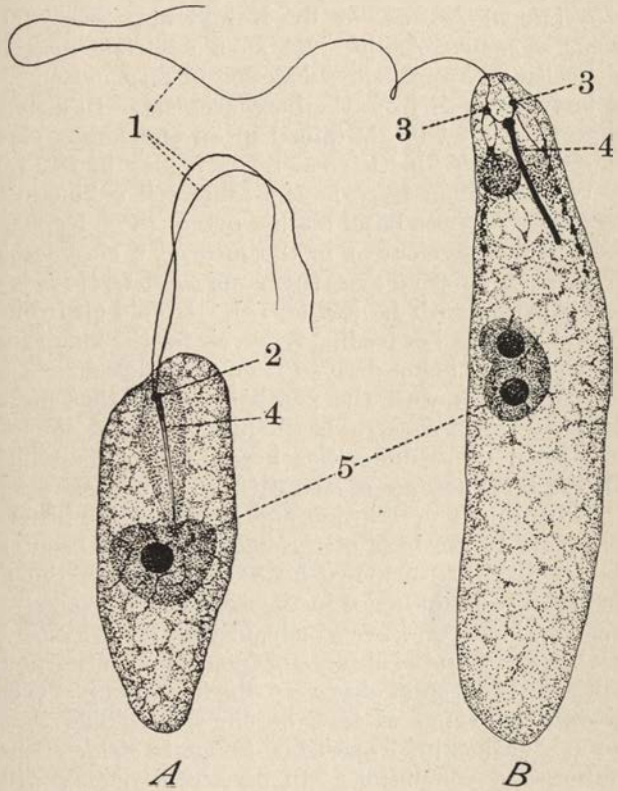

FIG. 46.—*A*, *Chilomonas paramecium; B*, *Peranema trichophora*, (1) flagella; (2) two fused blepharoplasts; (3) blepharoplast divided prior to division of the cell; (4) parabasal body; (5) nucleus. (After Calkins.)

flagellum (Fig. 45, *B*). Boeck (1921) has confirmed these findings. Or, the blepharoplast may migrate toward the posterior end of the cell where with or without division to form blepharoplast and basal body, it gives rise to a flagellum, which becomes the vibratile margin of an undulating membrane as in the majority of trypanosomes (Fig. 48, *E*). In still other cases the blepharoplast also gives rise to one endoplasmic fibril or rhizoplast, which extends deeply

into the cell as in *Chilomonas paramecium*, (or in *Rhizomastix* Mackinnon), or a number of such rhizoplasts may be formed as in *Mastigella vitrea* (Fig. 46). In these cases the blepharoplast divides independently of the nucleus at periods of cell division (Fig. 46, *B*).

2. *Parabasal Body and Blepharoplast.*— As a centriole may be contained in an endobasal body which consists largely of chromatoid substance, so may a basal body be enclosed in chromatoid substance of a blepharoplast, as shown by Goodey (1916) in the flagellate *Prowazekia* (*Bodo*) *saltans*, or by Kofoid and Swezy (1915) in *Trichomonas augusta*. Again, just as a centriole may be freed from its enclosing chromatoid substance in an endosome, so may the basal body be freed from the blepharoplast. In a similar way the blepharoplast may be contained in an embedding chromatoid mass of a cytoplasmic kinetic element, or it may be free from such a mass. We may then have in the same cell a kinetic complex consisting of one or more basal bodies, one or more blepharoplasts, and a residual kinetic element in the form of a chromatoid mass. To this residual chromatoid mass the name *parabasal body* is applied, the term originating with Janicki (1915). Kofoid (1916) interprets its function as a storage or feeding reservoir for the kinetic elements, its substance in turn being derived from the nucleus.

It is in connection with the parabasal body that most of the difficulties have arisen concerning the interpretation of cytoplasmic kinetic elements. The difficulties began with Schaudinn's work (1904) on the trypanosome of the little owl (*Glaucidium* [*Athene*] *noctuæ*). Schaudinn's description and figures of the history of the kinetic elements at the base of the flagellum have been cited and copied in practically every text-book dealing with the Protozoa and have had a wide influence in theoretical protozoölogy. Other keen observers, however, have sought in vain for evidence corroborating this history. In the absence of such confirmation and in view of the multitude of different observers who find a simpler explanation in many different types of trypanosomes, including that of the little owl (see Minchin, Robertson, Sergent, *et al.*), Schaudinn's interpretation and conclusions can be accepted only with many reservations.

The essential point in Schaudinn's description was the origin by heteropolar mitotic division of the nucleus of a recently fertilized cell (?), of a larger nucleus which becomes the nucleus of the cell, and a smaller nucleus which forms the kinetic complex. This smaller nucleus divides again by mitosis, also heteropolar, the smaller portion becoming the basal granule which forms the flagellum and the "myonemes" of the undulating membrane, while the larger portion remains intact as a homogeneous deeply-staining granule. The contested points in regard to this phase of Schaudinn's work are, first, the "fertilized cell" of the trypanosome, which is now

generally regarded as a stage in the life history of an entirely different parasite of the little owl (Minchin enumerates no less than five different types of protozoön parasites which may live simultaneously in the blood of this owl). A second contested point is the origin of the kinetic elements of the cytoplasm by mitosis. Other contested points and untenable conclusions drawn from them have to do with sex differentiation and parthenogenesis which need not be considered here.

It is not at all impossible that Schaudinn may have seen the emergence of a kinetic element from the endosome of the nucleus as described above in the case of *Nägleria gruberi*, and the similar emergence of a basal granule or blepharoplast from a chromatoid mass in the cytoplasm. The interpretation of such possible stages as mitotic nuclear division, and the smaller products of such division as nuclei, has led to numerous theoretical developments which have only a narrow basis of fact. Two years after Schaudinn's paper appeared, Woodcock translated it into English and conferred the name "kinetonucleus" on the smaller body resulting from the heteropolar mitotic division, and the name "trophonucleus" on the nucleus of the cell. Schaudinn himself was the first to announce this binucleate character of the trypanosome body and the hypothesis was taken up by his followers, Prowazek, and notably Hartmann (1907). The latter developed the conception into an elaborate view of original nuclear dualism upon the basis of which he created a special group of the Protozoa including trypanosome-like flagellates and hæmosporidia, which he called the "Binucleata."* As Doflein points out, not only do the hæmosporidia have no blepharoplasts as do the trypanosomes, but blepharoplasts in the latter are not to be considered nuclei. In this use of the term blepharoplast Doflein includes the structure to which Woodcock gave the name kinetonucleus, but he employs the term in a special sense as a kinetic element, while German writers generally use it for structures of widely different significance. Thus Schaudinn, although convinced of its nuclear character, nevertheless called it a blepharoplast. French writers as a rule speak of it as a centrosome (*e. g.*, Mesnil, Laveran, etc.) as do some English observers (*e. g.*, Moore and Breinl); many of the latter, however, follow the original nuclear interpretation, Bradford and Plimmer following Stassano, regarding it as a "micronucleus" and comparing it with the smaller nucleus of the ciliates, while Woodcock and Minchin considered it a "true nucleus."

The essence of the problem indicated by the various usages of these familiar terms comes down to a decision as to whether the so-called kinetonucleus, by which is meant the relatively large

* For critiques of the Binucleata, see particularly Minchin (1912), Dobel (1911.)

chromatoid body in the cytoplasm and closely connected with the basal granule, is a nucleus, or a kinetic center of the cell, or neither. Woodcock's term connotes a happy combination of both nuclear and kinetic possibilities; the kinetic function evident from its relation to basal granules or blepharoplasts, while its nuclear characteristic is seen mainly in the deeply-staining chromatin-like substance of which it is composed as well as by its frequent connection with the nucleus. Some writers, notably Rosenbusch (1909), giving free play to the imagination, and under the conviction that it is a nucleus, describe it as such, with centriole, "karyosome," nuclear space which may contain chromatin granules, and a nuclear membrane. The extremely minute size of this organoid and the pranks which the Romanowsky stain or any of its modifications may play with it, as they do with structures of the actual nucleus, together with a fertile imagination, are sufficient to account for the perfect nuclear type which Rosenbusch, for example, describes. Other observers, while maintaining its nuclear character, do not accept this extreme interpretation; Minchin, for example, describes it as a "mass of plastin impregnated with chromatin staining very deeply, rounded, oval, or even rod-like in shape" (Prot. p. 288).

If we bear in mind the many types of granules in the cell which stain like chromatin with certain dyes, it seems unnecessary, to say the least, to make the term nucleus, which stands for a well-known and easily recognized organoid of the cell, elastic enough to embrace cytoplasmic bodies in regard to which there is so little evidence of nuclear structure or nuclear function. In well fixed and stained material the so-called kinetonucleus affords little evidence of nuclear make-up; it appears as a homogeneous mass of chromatoid material which divides into equal parts prior to division of the nucleus. Such features do not make it a nucleus any more than similar features make nuclei of pyrenoids, or of other plastids of the cell. Functionally, and unlike the nucleus, it is not necessary for the vital activities of the organism, as shown by the experiments of Werbitski (1910), confirmed by others, in which by the use of certain chemicals the "kinetonucleus" of *Trypanosoma brucei* disappears without any effect upon the movements and reproduction of the trypanosome, a race being formed in which this organoid is absent. Nor can the "kinetonucleus" be regarded as a centrosome, for although closely connected with basal granules, it never behaves like an attraction center (see Fig. 47, p. 96). With the exception of Schaudinn's account and the overdrawn account by Rosenbusch there is no evidence that it divides by mitosis; it never develops chromatin which by any stretch of the imagination can be called chromosomes.

If the "kinetonucleus" is not a nucleus nor an active kinetic center of the cell, then any misleading appellation such as kinetonucleus, centrosome, or blepharoplast, which indicates co-partnership with

the actual cell nucleus or other easily recognizable organoid, should be discarded together with the supplementary term trophonucleus. The group Binucleata is sufficient evidence to show how far afield we may be led by conceptions indicated by such misleading names. Among names suggested to replace the term kinetonucleus is "kinetoplast" used by Wenyon, Dobell, and Alexeieff, and "parabasal body" (Janicki) as used by Kofoid.

The non-committal term parabasal body was first employed by Janicki (1915) to designate an accessory structure in the kinetic complex of *Lophomonas* (Fig. 98, p. 212). Analogous structures have since been found in practically all of the parasitic flagellates thus far described, although not found in free-living types generally. It is present as a globular mass of deeply-staining substance close to the blepharoplasts of types like *Trypanosoma brucei*, *Bodo edax* or *Bodo lacertæ* (Fig. 38); as an elongate mass in most of the *Cryptobia* species (Fig. 47, *C*); as a long basal filament in *Trichomonas augusta* (Fig. 72, p. 139); or *Chilomastix mesnili;* as a spirally coiled mass in *Devescovina striata* (Fig. 47, *F.*), etc. It apparently differs in size and form in different phases of the same organism as in *Bodo lacertæ* where, in addition to the globular form, it may be rod-like or partly coiled or absent altogether. In *Chilomastix mesnili* an homologous rod-like body, termed the *parastyle,* arises from a second blepharoplast (Kofoid and Swezy, 1920. Fig. 45).

The most extensive work on the parabasal body has been carried out by Kofoid and his followers who regard this structure not as a nucleus nor as a kinetic center, but as a "kinetic reservoir" or a reservoir of substances which are used by the animal in its kinetic activities under the conditions of its dense environmental medium. This substance, according to Kofoid, appears to form at the expense of the nuclear chromatin and increases or decreases—that is, the parabasal body becomes larger or smaller apparently in relation to metabolic demands. When the parabasal body is poor in chromatin the blepharoplast and nucleus may be rich and *vice versa.* "Our data are too incomplete to give a clear picture of the process, but as far as they go they suggest the origin of the parabasal at the expense of the chromatin of the nucleus, the movement of stainable substance on the rhizoplast, either to or from the blepharoplast at the base of the flagella, and the wax and wane of the parabasal" (Kofoid, 1916, p. 5).

Kofoid's interesting and suggestive interpretation of the nature of the parabasal is very well sustained by the morphological relations of blepharoplast, nucleus and parabasal body in widely divergent types of flagellates. Morphologically, a series representing a gradually increasing complexity is illustrated by (1) *Nägleria gruberi,* in which the blepharoplast arises by division of the intranuclear kinetic center and remains connected with it by a centro-

desmose or, in this case, a cytoplasmic rhizoplast; (2) *Scytomonas subtilis* in which the blepharoplast is not connected with the nucleus and gives rise only to the flagella; (3) *Bodo edax*, or species of *Cryptobia* in which a large chromatoid mass, the parabasal body, is connected by rhizoplasts with the blepharoplast, or may be independent of it; (4) *Bodo lacertæ* in which basal bodies arising from the

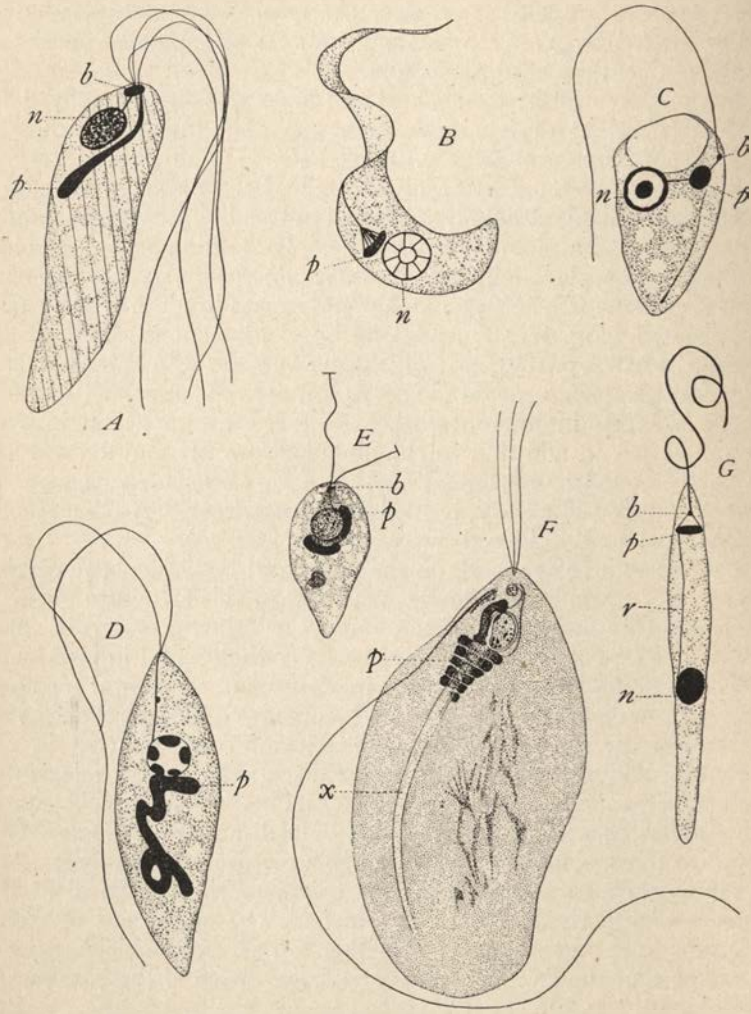

FIG. 47.—Types of parabasal body. *A*, *Polymastix; B*, *Trypanosoma cruzi; C*, *Cryptobia* sp. *D*, *Bodo lacertæ; E*, *Prowazekia* sp; *F*, *Devescovina striata; G*, *Herpetomonas musca-domesticæ*. (*b*) Blepharoplast; (*p*) parabasal body; (*n*) nucleus; (*x*) axostyle. (*A*, *C*, *D*, *G*, after Swezy; *B*, after Chagas; *E* and *F*, after Doflein.)

blepharoplast, blepharoplast, and parabasal body, are all independent; (5) *Giardia augusta*, in which the independent blepharoplast, basal bodies, and parabasal body, are all double and arranged in perfect bilateral symmetry; (6) *Calonympha grassii* (Fig. 49), in which nuclei, parabasal bodies, blepharoplasts and basal bodies are

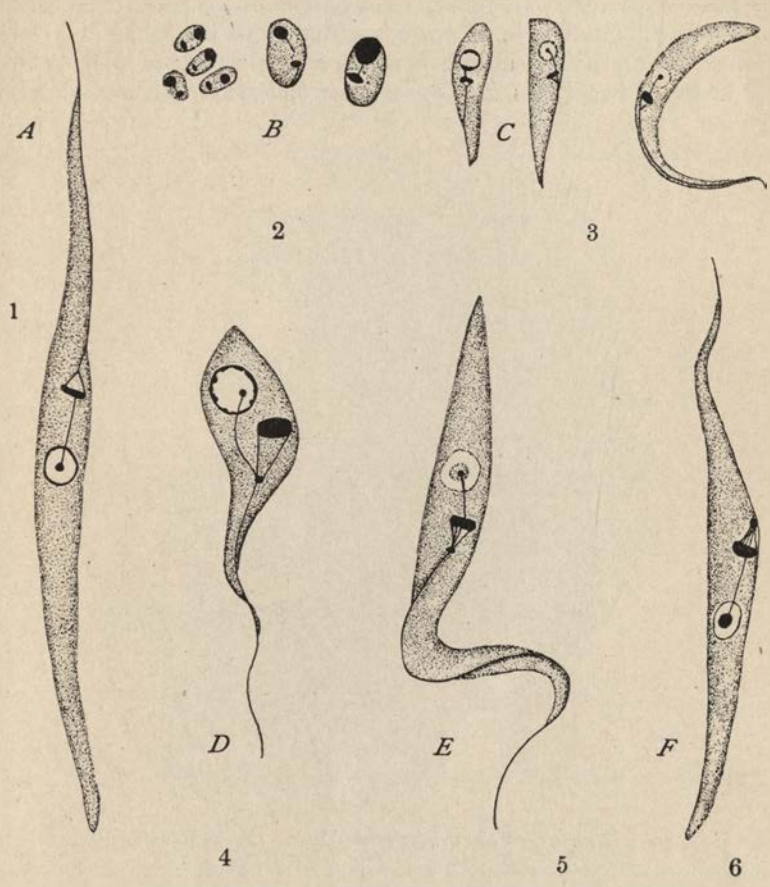

FIG. 48.—Relation of parabasal to nucleus. *A*, *Crithidia euryophthalmi* endosome of nucleus and parabasal connected by rhizoplast; *B*, origin of parabasal from endosome of nucleus; *C* and *D*, differentiation of parabasal and rhizoplasts; *E*, *Trypanosoma cruzi*, and *F*, *Crithidia leptocoridis* for comparison. (After McCulloch.)

multiple and in which axial threads (rhizoplasts) unite to form a central axial supporting rod; (7) *Trichonympha campanula* in which the blepharoplast (centroblepharoplast) acts as a centrosome in mitosis while long rhizoplasts connecting distal basal bodies with the blepharoplast form a complex radial system of astral rays (Figs. 47 to 51).

In many cases the blepharoplast, which is the central element of the kinetic complex, remains connected with the nucleus by a rhizoplast as a permanent record of the intranuclear origin of the entire complex (Fig. 47). In many cases the blepharoplast is double, as in most biflagellated forms (Fig. 46, *A*); in others it is triple, as in *Trimastigamœba philippinensis* or *Chilomastix mesnili* (Fig. 45, *B*); in some it is quadruple, or contains four basal bodies as in *Trichomonas;* in others it is multiple, forming a ring of blepharoplasts about a bundle of flagella as in *Lophomonas blattarum* (Fig. 98, p. 212).

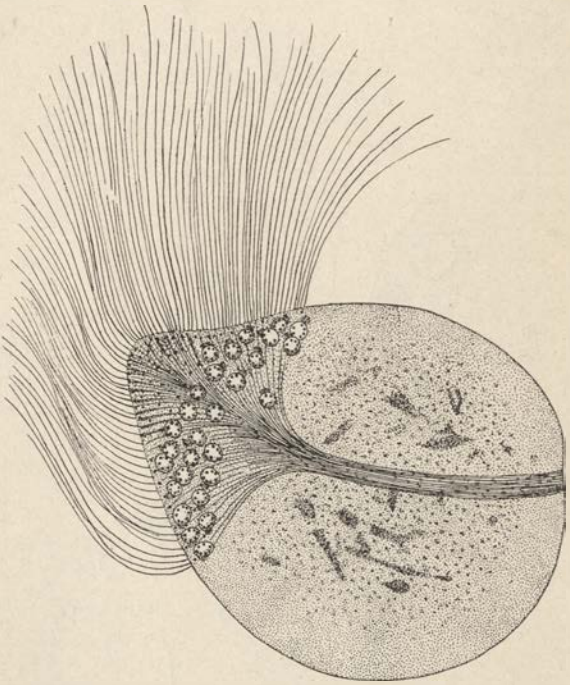

Fig. 49.—*Calonympha grassii* Foa. (From Doflein.)

Finally in flagellates with multiple nuclei (family *Calonymphidæ*), in addition to a number of free blepharoplasts and parabasal bodies, each nucleus is accompanied by a blepharoplast which gives rise to three uniform flagella and one longer, band-formed flagellum, by a parabasal body, and by a rhizoplast (axial thread, Fig. 49).

Many of these aggregations of kinetic elements are sufficiently complex to justify the term neuromotor system of Sharp and Kofoid and appear to form a coördinated whole as shown by the reaction after maceration when they retain their connections and remain together for some time after the supporting protoplasm has disap-

peared (*Trichomonas*, Kofoid). The term is certainly justified in connection with the remarkable kinetic structures of flagellates belonging to the family Trichonymphidæ. In *Trichonympha campanula*, Kofoid and Swezy (1919) describe the system as composed of an external coating of cilia-like motile organs, three zones of flagella with their basal bodies, rhizoplasts connecting basal bodies with a great anteriorly placed blepharoplast, and more deeply-lying myonemes which apparently are not connected with the blepharoplast (Fig. 50). Kofoid and Swezy regard the central organoid as a kind of superblepharoplast, calling it the "centroblepharoplast" since it has the attributes of a centrosome. When it divides the entire

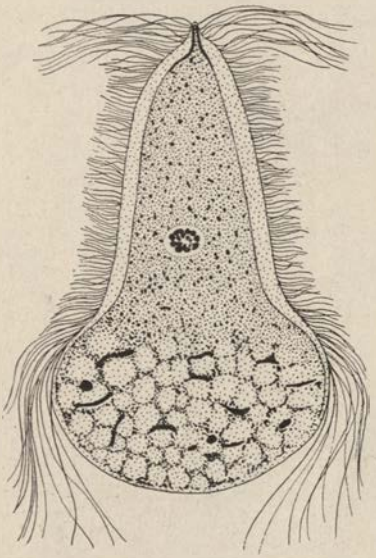

FIG. 50.—*Trichonympha campanula* Kof. and Swez. (After Kofoid and Swezy.)

aggregate of kinetic elements of the cortical zone divides with it, forming a mitotic figure with centrosomes, central spindle and astral rays (Fig. 51). The connecting fibrils of the centrosomes, unlike the centrodesmose in Metazoa, remain outside of the nucleus (as it does in many other flagellates) and is called the *paradesmose* by Kofoid to distinguish it from the centrodesmose or central spindle. Superficially, at least, this highly complicated type of mitotic figure resembles the division figure of *Noctiluca miliaris* in which the kinetic elements are also extranuclear throughout the entire process. Here, however, there is no such development of powerful motile organoids and the division figure is relatively simple (Fig. 52).

As specially modified parabasal bodies, finally, may be included

peculiar deeply-staining rod-like bodies (Staborgan) of *Peranema trichophora* (Fig. 46, *B*), of *Anisonema*, *Entosiphon*, and related genera, which behave like a parabasal in division and belong to the group of kinetic elements.

From this review of the cytoplasmic kinetic elements in the flagellates it is apparent that in endobasal bodies, basal bodies, and parabasal bodies we have to do with structures closely connected

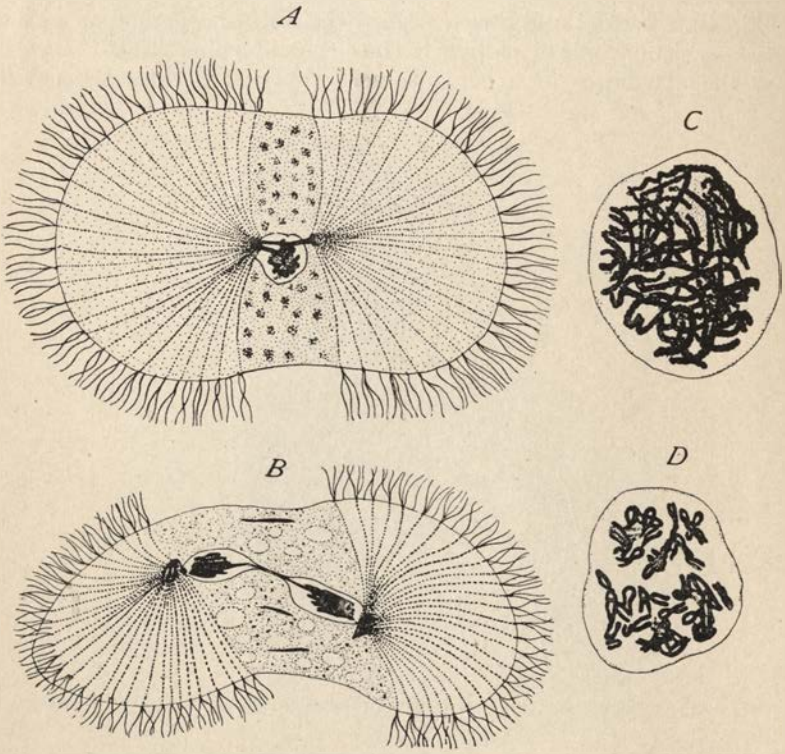

FIG. 51.—*Trichonympha campanula* in division. *A*, and *B*, prophase and anaphase of nuclear division; the divided centroblepharoplast forms the poles of the spindle and are connected by a paradesmose. *C*, and *D*, breaking up of chromosome spireme into chromosomes which show a tendency to unite in pairs. (After Kofoid and Swezy.)

with the kinetic activities of the organism and closely related to each other. The chromatoid substance of which they are composed may or may not be chromatin, although the evidence adduced indicates that it arises from the nucleus and is similar to chromatin in its staining reactions. It does not behave like chromatin during division of the cell, but like pyrenoids, or chromatophores, where each granule reproduces its like by division; nor does it afford any

evidence of constructive metabolic activities in the cell. For these reasons I believe, with Kofoid, that the term "parabasal body" expresses the relationships and functional activities of the so-called "kinetonucleus" much better than does the latter term and should take its place in literature dealing with the Protozoa.

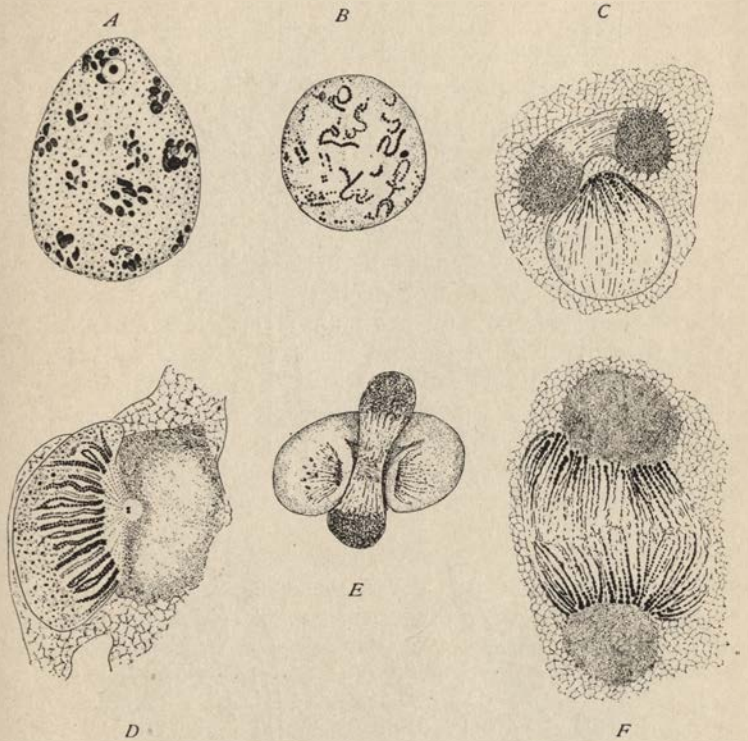

FIG. 52.—*Noctiluca miliaris* Sur. Origin of the spindle and division of the nucleus. *A*, Endosomes fragmenting; *B*, grouping of fragments into linear chromosomes; *C*, formation of the amphiaster and orientation of the chromosomes; *D*, section through one pole of anaphase stage showing centrioles and mantle fibers; *E*, nuclear furrow holding the amphiaster; *F*, anaphase stage. (After Calkins.)

3. *Other Cytoplasmic Kinetic Elements.*—A unique cytoplasmic kinetic element, apparently homologous with the centroblepharoplast of certain flagellates, is found in some types of Heliozoa. The non-committal name central granule (Centralkorn) was given to this structure by Grenacher, in 1869, who was the first to observe it. In some types it lies in the geometrical center of the cell (*Acanthocystis aculeata, Sphœrastrum fockei, Raphidiophrys pallida,* etc.); in other types it is excentric (*Dimorpha mutans, Wagnerella borealis*) or absent altogether (*Actinophrys sol, Actinosphœrium eichhornii,*

Camptonema nutans, etc.). In the ordinary vegetative activities of the cell, radiating fibers starting from the central grain extend through the protoplasm to the periphery, where they form the axial filaments of the pseudopodia (Fig. 53). In division stages of the

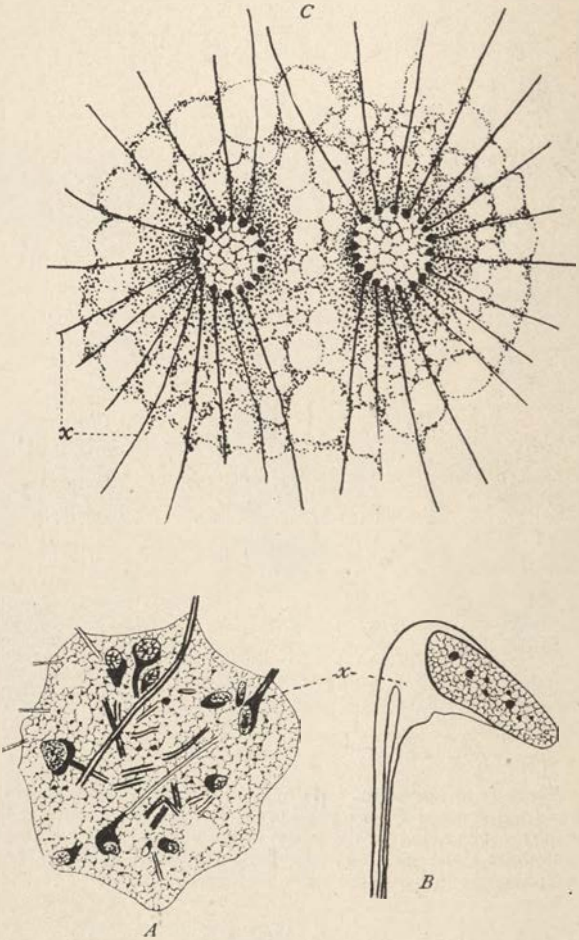

FIG. 53.—Relation of axial filaments to nuclei. *A* and *B*, *Camptonema nutans* with nuclei partly embedded in the substance of the axial filaments; (*x*) axial filament; *C*, section of *Actinophrys sol* with axial filaments arising from intranuclear granules in recently divided nuclei. (After Schaudinn.)

cell, the central grain first divides forming an amphiaster consisting of centrosomes, centrodesmose and astral rays made up of the radiating fibrils (Fig. 60—see also *Trichonympha campanula*). The central grain, however, takes no part in reproduction by budding,

whereby amœboid or flagellated buds are formed which contain a nucleus derived from the parent cell nucleus, but no central grain. This nucleus, however, contains an endobasal body which divides and one of the daughter granules emerges from the nucleus as it does in *Nägleria gruberi* (p. 86), but retains its centrodesmose for some time and ultimately forms the central grain of the adult organism (Schaudinn (1896), Zuelzer (1909), *Acanthocystis aculeata*, *Wagnerella borealis*, Fig. 60). Similarity with the centroblepharoplast in flagellates is thus shown (1) by its origin from an intranuclear centriole; (2) by its relation to axial filaments which are homologous with rhizoplasts; (3) by its history during mitosis. The analogy is further strengthened by its relation to the flagella and to the axiopodia which are simultaneously present in some of the Heliomonadida (*Actinomonas mirabilis*, Kent, *Ciliophrys marina*, Caullery, and *Dimorpha mutans*, Gruber). In *Dimorpha mutans*, the central grain lies near one pole of the cell where it forms the basal body of the two flagella as well as the focal point for the axial filaments, here flagella and axial filaments appear to be homologous structures. According to Zuelzer the pseudopodia of *Wagnerella borealis* are withdrawn at times owing to the contraction of the entire complex of radiating fibrils, and basal bodies lying at the bases of the axopodia become grouped in a zone of granules about the central grain. When the pseudopodia are again formed the granules migrate centrifugally to the periphery and, as basal bodies, give rise to the axial filaments.

In Heliozoa without a central grain the axial filaments in some cases center in the nucleus in which there are many distinct and definite granules of uniform size distributed about the outer zone, from each of which an axial filament appears to rise (Fig. 53, *C*). In *Camptonema nutans* the nuclei are multiple, and, according to Schaudinn, each one gives rise to a single pseudopodial element (Fig. 53, *A*), but in *Actinosphærium eichhornii*, which is also multinucleate, the axial filaments apparently have no connection with either nuclei or central kinetic elements.

Apart from kinetic elements like centroblepharoplasts which, at the same time, are centers of mitotic activity of the nucleus and of kinetic activity of the motile organs, there are comparatively few examples of kinetic elements comparable with centrosomes of Metazoa. They are best represented in non-motile organisms such as Sporozoa, whereas in freely-moving types there is always some peculiar feature which makes the homology with centrosomes doubtful.

The most frequently cited example of a centrosome in Protozoa was first described by Hertwig in the case of *Actinosphærium eichhornii* (Fig. 64). Here, during the formation of the first maturation spindle minute granules of chromatoid substance are cast out of the nucleus and condensed into one or two minute centrioles from

which fibrillar structures radiate into the cytoplasm and throughout the nucleus. This structure, however, has no permanent relation to the cytoplasm or nucleus, but disappears after the first maturation spindle is formed while subsequent maturation spindles and spindles of division stages are characterized by pole plate formation (see p. 81). Much more typical centrosomes are found by Arndt (1924) in *Hartmannella* (*Pseudochlamys*) *klitzkei* (Fig. 41, p. 85) and in the Gregarinida, especially in the *Monocystis* types where they have been described by Léger, Brasil, Mulsow, Doflein and others. In *Monocystis rostrata*, for example, a single centrosome with marked astral radiations, lies outside the nuclear membrane (Fig. 63). An amphiaster is formed as in egg cells of Metazoa, and a complete mitotic figure results. Similar centrosomes occur in *Urospora lagidis* St. *Gonospora varia*, Léger and *Stylorhynchus longicollis*, St.

Transitory centrioles and centrospheres are present in *Noctiluca miliaris* Sur. In resting stages there is no evidence of the centrioles while the centrosphere disappears in the granular mass of endoplasm. In the early stages of cell division, however, the centrosphere condenses into a fairly homogeneous cytoplasmic mass of large size outside the nucleus. This divides into two daughter spheres connected by a fibrillar centrodesmose, while centrioles of unknown derivation appear in each sphere and are connected by mantle-fibers with the ends of the chromosomes (Fig. 52).

The very peculiar Nebenkern of the rhizopod *Paramœba eilhardi* as described by Schaudinn and the "nucleus secundus" of *P. pigmentifera* and *P. chætognathi* as described by Janicki, are less easily homologized with centrosomes. They have nothing to do with the nucleus during division nor with motile organs, but, as Doflein hints, may be interpreted as parasites from their appearance in Janicki's figures.

In general we do not find the same types of kinetic elements in Infusoria that are found in other forms of Protozoa. Blepharoplasts, parabasal bodies and centrosomes are still unknown in ciliates, although certain peculiar kinetic elements are present here which may turn out to be homologous with one or more of these structures. Endobasal bodies, however, are known in micronuclei of a few types (*e. g.*, *Uroleptus mobilis*, *Oxytricha fallax*, and in some macronuclei (*e. g.*, *Chilodon cucullus*, Fig. 34, p. 77). On the other hand, certain special types of cytoplasmic kinetic elements such as myonemes, motorium, and conductile fibers, are characteristic of the ciliates some of which become highly complicated coördinated neuromotor elements.

The most widely distributed of the kinetic elements are the basal granules of the cilia, which are situated in the contractile zone of the cortex (see p. 144). The exact nature of these extremely minute bodies is unknown and their origin or renewal is purely hypothetical.

Collin (1909) and Entz (1909) record some observations which suggest their derivation from nuclei (Entz) or at least some connection with them (Collin). A single basal body gives rise to a single cilium (Fig. 54) but groups of them are found at the bases of the more complicated membranes, membranelles and cirri the number varying with the species. Thus Maier describes 2 in the membranelles of *Nyctotherus cordiformis* and many of them arranged in a row in the membranelle of *Stentor niger;* in undulating membranes of the vorticellids Maier and Schröder describe 3 rows of basal granules while in the "paroral" and "endoral" membranes of *Glaucoma scintillans* there are 5 and 10 rows of basal granules

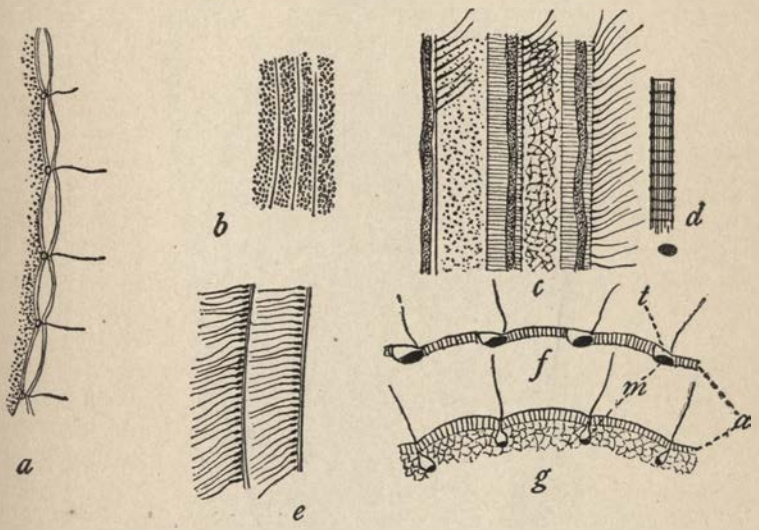

FIG. 54.—Cilia and myonemes of Infusoria. *a*, Membrane and periplast of *Stentor cæruleus; b*, c, and *e*, rows of cilia of same; *d*, myoneme of same; *f*, optical section of membrane and myonemes of same, and *g*, optical section of cortex of *Holophrya discolor*. (*a*, *b*, *e*, after Johnson; *c*, *d*, *f*, and *g*, after Bütschli.)

respectively (Maier). In the cirri of *Stylonychia histrio* which are circular in cross-section, according to Maier, there is a discoidal plate of basal bodies. Alverdes (1922) found that an isolated cilium will beat if the basal body is attached, not otherwise.

Myonemes.— One of the most striking characteristics of certain types of ciliates is their power of contraction. A fully-expanded *Spirostomum ambiguum* may be 2 mm. in length but, on irritation, it suddenly contracts to one-quarter that size, or a *Trachelocerca phœnicopterus* contracts to one-twelfth its original length (Lebedew); a *Folliculina ampulla* with its great peristomial lobes widely outspread quickly folds itself completely into its comparatively narrow tube (Figs. 84, 165), or an entire colony of widely distended indi-

viduals of *Zoöthamnium arbuscula* contracts instantly into a minute ball. These varied movements which are quite independent of movements of translation or rotation, are due to the contraction of specialized muscle-like fibrils, the myonemes. These are long,

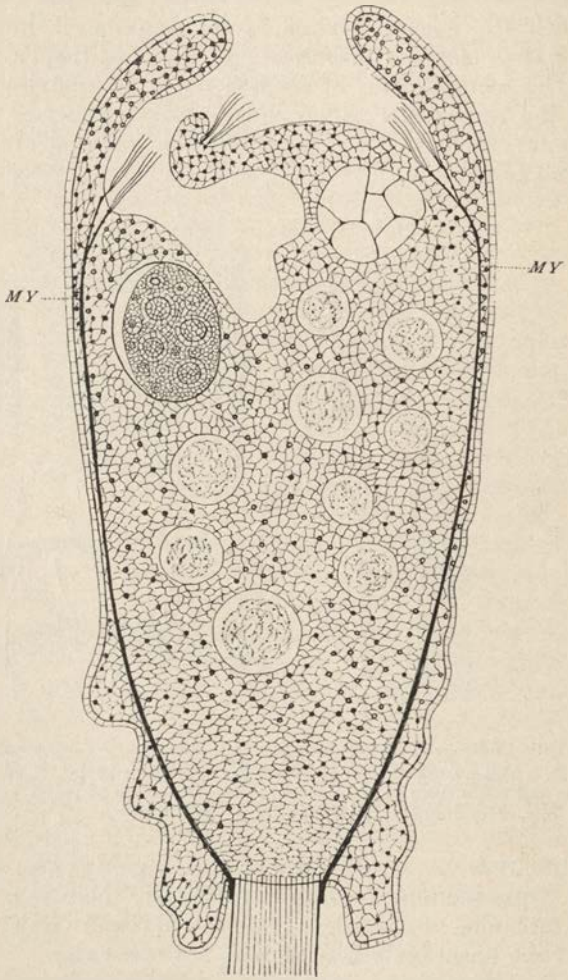

FIG. 55.—*Epistylis plicatilis;* longitudinal section showing myonemes (*MY*) from membranelles to base of cell. (After Schröder.)

delicate, contractile threads, circular or band-like in cross-section situated in the cortical zone and running throughout the entire length of the body either straight (*Stentor*) or spirally (*Spirostomum*). In some cases a second set of myonemes run transversely about the body as in the peristomial regions of *Campanella umbellaria*

or various species of *Stentor*. The myonemes of *Stentor cœruleus* or *Prorodon teres* lie in characteristic canals, which appear hyaline in contrast with the granular adjacent "ribs" of the ectoplasm. Their finer structure has been made out in only a few types, in *Stentor cœruleus* perhaps better than in any other. Here Schröder describes a typical cross-striping due to alternate rows of light and dark substance (Fig. 54 *d*.)

In the majority of cases the contractile effect of the activity of myonemes is possible only by their intimate connection with the

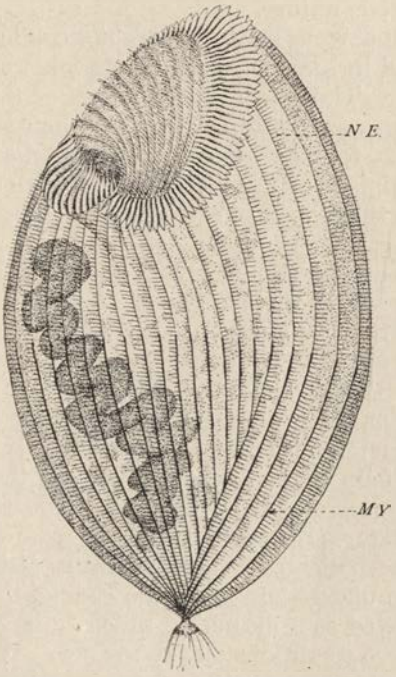

FIG. 56.—*Climacostomum* sp. To show neurophanes (*NE*.) and myophanes (*MY*). (Original.)

firm membranous cortex which encloses the entire animal, a connection which makes it possible for a coördinated contraction of the whole animal at once. A retraction of special regions of the organism involves the attachment of one end of the contractile element to some relatively fixed structure, as muscles in vertebrates are attached to the endoskeleton (Fig. 55). In many cases the general cortex serves this purpose as in the sphincter-like myonemes of the Vorticellidæ (Schröder), or the retractile elements of the "seizing organ" or "tongue" of *Didinium nasutum* (Fig. 89, p. 180), or the closing apparatus of the operculum-bearing types of ciliates. In

some cases, however, especially in parasitic ciliates like *Ophryoscolex* or *Diplodinium ecaudatum*, there is a specialized differentiation of the "cuticle" discovered by Gunther and well described by Sharp. These peculiar differentiations function according to the latter observer, as endoskeletal structures for the attachment of conspicuous band-form myonemes, which serve as retractor strands for drawing into the body a characteristic gullet and adjacent organoids (Fig. 2, p. 20). These skeletal elements are formed from the ectoplasm and are hardened, according to Eberlein, by a deposit of silicic acid which, as Sharp implies, may be the explanation of their rigid but brittle nature.

Myonemes or analogous organoids are not confined to the ciliates but may be found in some types of Gregarinida and in one group of the Radiolaria. The so-called myonemes of the Trypanosomatidæ, however, are very doubtful kinetic elements but, more probably, are analogous to the cuticular markings which are frequently found on the periplast of flagellates. In some of the gregarines, myonemes form a thick layer of extremely fine fibrils in the contractile zone of the ectoplasm, running circularly, or possibly spirally, about the cell, their contractions giving rise to the peristaltic movement so characteristic of these forms.

Myophrisks of the Radiolaria are contractile strands which are fastened by their distal ends to the extremities of the axial bars of the Acantharia. The proximal ends fray out into fibrils which are lost in the reticulum of the gelatinous mantle or calymma, of the ectoplasm. By their contractions the calymma is drawn up to the ends of the axial bars whereby the diameter of the organism is increased and its specific gravity decreased, the reverse occurring with their relaxation. The myonemes thus seem to play a part in the hydrostatic activities of these Radiolaria although this function is difficult to understand since the change in specific gravity is usually interpreted as a means by which these motionless forms escape from adverse conditions on the surface. We should expect, however, that rough water or other surface conditions deterimental to the organisms, would be sources of stimulation which should cause the contractile elements to contract and thus to defeat their apparent purpose by decreasing the specific gravity.

Coördinating Fibers.— If a single cilium of a resting *Pleuronema* be touched the entire organism responds. Here and in similar cases there appears to be a definite tactile function. In flagellates also it is not improbable that certain flagella, as the anterior flagella of *Caduceia theobromæ* described by França (1918), or indeed possibly all flagella have a more or less well-developed sensory function. In ciliates, such as *Paramecium caudatum*, with a uniform coating of cilia, the motile elements do not all beat simultaneously, but a wave of contraction, beginning at the anterior end, passes down the

cell to the posterior end. Cilia in the same transverse row beat synchronously, but each cilium in a longitudinal row begins its beat shortly after the cilium anterior to it has started and before it has ended its beat (Verworn). The cilia of transverse rows are thus synchronous, those of longitudinal rows metachronous in their contractions, a phenomenon which accounts for the wave-like movement of undulating membranes which are formed of fused cilia of longitudinal rows (well shown in the undulating membranes of the Vorticellidæ). According to Alverdes (1922) isolated cilia with basal body may act independently of a coördinating system but they do not react to stimuli.

This regularity of cilia movement which may be easily seen in the uniform ciliary coating of *Nyctotherus ovalis* from the cockroach, indicates the transmission of impulses and the activity of some coördinating mechanism in the cell. Entz, Maier, Schuberg and many other observers, have found distinct fibers connecting the basal bodies of Protozoön cilia and have generally interpreted them as myonemes. Since forms like *Nyctotherus*, *Frontonia*, *Paramecium*, etc., which do not contract, show the same rhythmical action of the cilia, it is probable that the threads connecting their basal bodies are not myonemes but coördinating fibrils (Fig. 57). It is conceivable, moreover, that myonemes in a generalized condition may be both coördinating and contractile in function. In some cases, however, two distinct sets of fibrils have been observed, one of which is interpreted as contractile, the other as conductile. Thus Neresheimer described "myophanes" and "neurophanes" in *Stentor cæruleus*, and *Climacostomum virens*, the former extending the entire length of the body, the latter only from the base to the center (Fig. 56). On *a priori* grounds, it would seem that, as Yocom points out, Neresheimer made an unfortunate application of his two terms, his neurophane fibers, for example, to which he ascribes a transmitting function, being situated in the least advantageous position for the functions of irritability or conductility, Jennings having shown that the first and most strongly marked reactions to certain stimuli in ciliates appears in the anterior region, a result confirmed by Alverdes (1922).

The more recent observations of Sharp, Yocom, and Taylor, all from Kofoid's laboratory, afford more striking evidence of specific conducting or coördinating fibrils in ciliates. In connection with *Diplodinium ecaudatum*, Sharp described, for the first time in the literature, a system of connected fibrils emanating from a common mass of differentiated protoplasm, which he called a "motorium," the whole system being termed the "neuromotor apparatus." The motorium is situated in the ectoplasm of the anterior end of the organism between the two zones (adoral and dorsal) of membranelles (Fig. 2, p. 20, and Fig. 57). From it as a center a number of

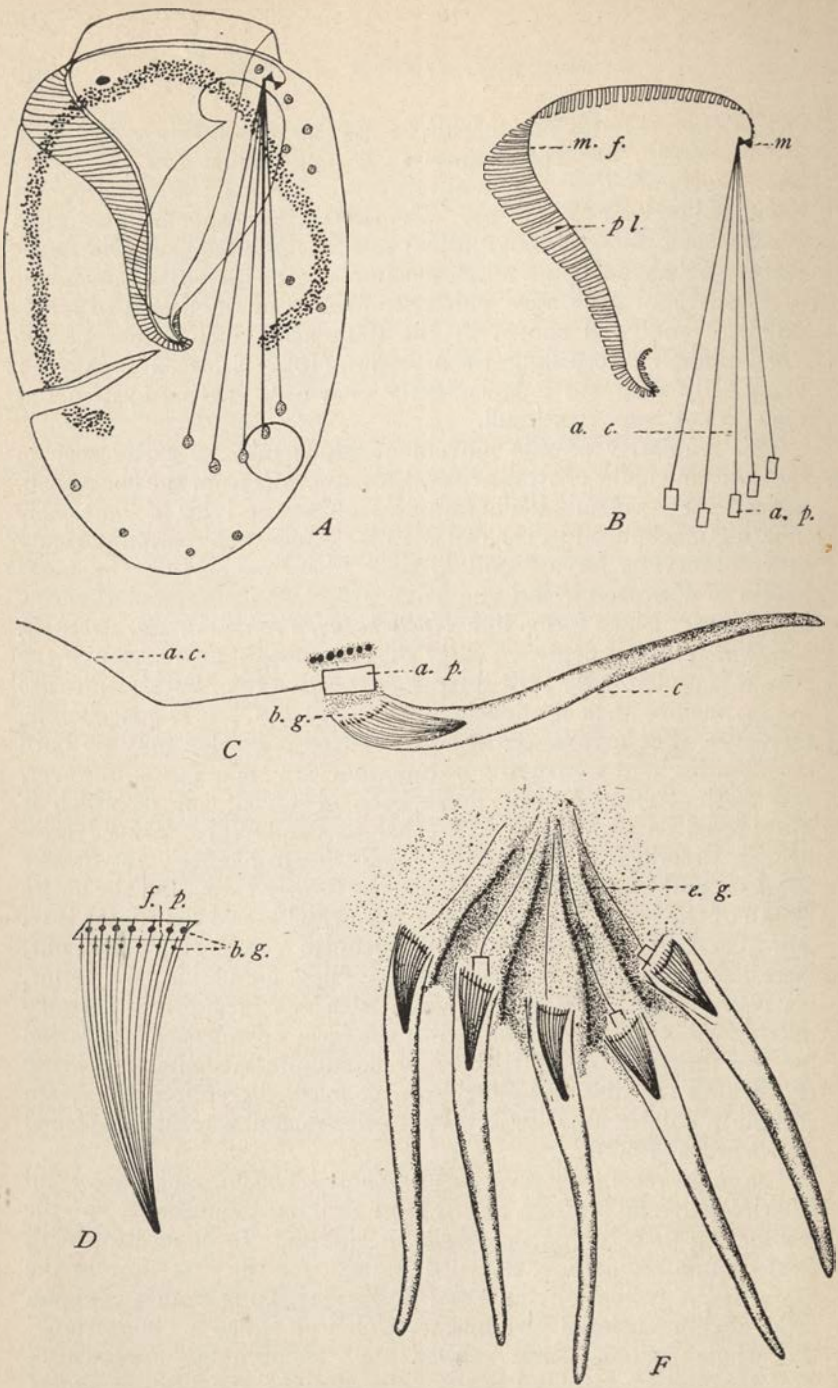

FIG. 57.—Micro-dissection of *Euplotes patella*. *A*, Individual with lateral cut; showing distribution of the cellular structures: *B*, neuromotor apparatus isolated; *C*, an anal cirrus with accompanying structures; *D*, an isolated membranella; *F*, the five anal cirri; (*a.c.*) anal cirri fibers; (*a.p.*) basal plates of the anal cirri; (*b.g.*) basal granules; (*c*) cirrus; (*e.g.*) ectoplasmic granules; (*f.p.*) fiber plate; (*m.f.*) membranelle fiber; (*m*) motorium; (*p.l.*) membranelle plates. (After Taylor.)

fibers pass to different regions of contractile activity. These fibers are named and interpreted by Sharp as: (1) A circumœsophageal ring strand (c. r. s.) running to a definite ring of substance similar to that of the motorium encircling the gullet (œs. ring), from which other fibers (œs. f.) apparently take their origin and run posteriorly along the retractile gullet; (2) a dorsal motor strand (d. m.) running to the bases of the adoral membranelles; (3) opercular fibers (op. f.) or a group of fibers running to the operculum (Fig. 2).

The delicacy of structure and the position of this amazingly complex aggregate are sufficient evidence to disprove any hypothesis of a supporting function. Self-perpetuation of the elements by division indicates no relationship to supporting structures such as trichites (oral basket) in the mouth regions of forms belonging to the family Chlamydodontidæ. Their position in the cell and the attachments of the several fibrils are arguments against their interpretation as myonemes.

McDonald (1922) has recently described a somewhat similar neuromotor system in *Balantidium coli* and *B. suis*. Here an anterior motorium gives rise to (1) a ring-form fibril which passes around the adoral cilia region and (2) a similar ring fibril passing around the gullet. Other elements of the system consist of basal granules of the cilia, from which rhizoplasts pass inward to the central region of the cell. At the point where each rhizoplast enters the endoplasm is a granular thickening from which a radial fibril passes toward the periphery where it ends blindly.

Evidence in favor of a conductile function of such a neuromotor system is furnished by the observations of Yocom (1918) and the micro-dissection experiments of Taylor (1920) on *Euplotes patella*. In Euplotidæ, apart from the motile organs, contractility is unknown, nevertheless the literature contains many references to myonemes in the several species. Distinct fibrils in these hypotrichs which Engelmann regarded as nerve-like in function, have been interpreted in the main as supporting or contracting elements (Maupas, Bütschli, Schuberg, Maier, etc.). Prowazek worked them out in some detail in the case of *Euplotes harpa* and Griffin (1910) in the case of *E. worcesteri*, both observers regarding them as contractile in function. Yocom has studied them more recently in *Euplotes patella* and a complex system, comparable with that of *Diplodinium ecaudatum* is described. A definitely staining bilobed mass of differentiated protoplasm which Yocom identifies as a motorium is situated in the ectoplasm near the right anterior angle of the triangular peristome (Fig. 57, *m*).

From one lobe of this mass a set of five prominent longitudinal fibrils which seem to emerge as a single strand, run to the bases of the five anal cirri near the posterior end (*a. c.*), from the other lobe a single fibril passes along the inner margin of the anterior lip and

down the left side of the peristome closely following the bases of the frontal and peristomial membranelles. In the anterior lip it gives rise to a simple network of branching fibrils (Yocom). The other cirri of the ventral surface are not thus connected with the motorium, and each appears to have an entirely independent set of fibers which run into the endoplasm and disappear in different directions.

Yocom attempted, rather unsuccessfully, to homologize the motorium with the blepharoplast of flagellates; until further observations are forthcoming in regard to the activities of this structure at different periods of cell life, it seems more expedient to regard the motorium as a structure peculiar to the ciliates than to add it to the already over-burdened conception of the blepharoplast.

The only direct evidence of the physiological nature of the neuromotor complex is furnished by Taylor's micro-dissection experiments with the same organism, *Euplotes patella*. Cutting the fibers connecting the anal cirri with the motorium had a noticeable effect on the normal reactions of creeping, swimming and turning, while severing the membranelle fiber led to characteristic irregularities in the usually coördinated activities of the membranelles and to abnormal spiral revolutions while swimming. Destruction of the motorium finally resulted in uncoördinated movements of the membranelles and of the anal cirri. This evidence, excellent as it is, rests upon an exceedingly delicate technic and upon the personal interpretation or estimation of minute differences between normal and induced reactions. It is a line of work, however, which invites further research and promises fruitful results.

4. NUCLEAR DIVISION AND THE PROBLEM OF CHROMOSOMES.

The aggregate of substances which have been described in the preceding pages make up living protoplasm. Each type of substance receives from the food supply, either directly or indirectly, materials for the up-building of its own type, the sum total of such processes constituting growth. Each type of substance, and each granule, grows to its limit of size and then divides, reproducing its like, a phenomenon which finds its visible expression in the division of pyrenoids, mitochrondria, basal bodies, blepharoplasts, parabasal bodies, nuclei, and finally the cell itself. Reproduction of the cell of a protozoön thus involves reproduction of all its parts.

The nucleus is the most complex of the formed organoids of the cell and its reproduction involves growth and division of its different elements. These may be more or less independent in their division, or they may be united in various simple or complex combinations during the division processes. Or the nuclear elements may be combined with extranuclear, cytoplasmic elements to form a char-

acteristic division figure representing the most highly perfected mechanism for the equal distribution of the more important cell elements which are thus perpetuated from generation to generation by equal division. Such a perfected mechanism, termed a karyokinetic or mitotic figure, is characteristic of nuclear division in cells of the Metazoa and of higher plants, the combination of processes whereby the constituent parts are equally distributed to daughter cells being known as indirect division, karyokinesis, or mitosis. Such processes involve division of centrioles and centrosomes, formation of a fibrillar spindle figure, dissolution of the nuclear membrane, aggregation of chromomeres into compact chromosomes which are identical in size, shape and number in corresponding cells of all individuals of the same species, and the longitudinal division of each chromosome in all somatic cells, separation of the daughter chromosomes and reconstruction of the daughter nuclei. In all Metazoa the processes of mitosis differ only in minor details and mitosis is the characteristic type of nuclear division, although direct division, whereby the nucleus divides without the formality of centrosomes and spindle or chromosome formation is known in a few cases.

In Protozoa, on the other hand, there is no one type of nuclear division common to all forms. Here we find gradation, in the association of constituent nuclear and cytoplasmic kinetic elements during division resulting in an enormous variety of division types. These vary in complexity from a simple dividing granule to mitotic figures as elaborate as in the tissue cells of higher animals and plants. Some observers see in these diverse types a possible evolution of the mitotic figure of Metazoa and use them as one would use the separate pieces of a picture puzzle to reconstruct its past history in development. Terms like "promitosis" (Naegler), "mesomitosis" (Chatton) and "metamitosis" (Chatton) may serve a useful purpose to indicate general types of the association of nuclear and cytoplasmic elements during division, but when an effort is made to give a specific name to each step in an increasingly complex series the result is a confusion of terms which defeats the useful purpose intended. Thus Alexeieff proposes a large number of specific names, not all his own, it is true, for protozoön division types which he regards as sufficiently definite to permit of recognition.*

Because of the multitude of diverse types of division figures in the Protozoa the difficulty of treating them in any general way has been admitted by all students of cytology as well as by protozoölogists. I shall endeavor here to convey an idea of this diversity and at the same time to describe some of the more frequent types of division figure without confusing the issue still more by my own views as to

* These terms include Promitosis, Proteromitosis, Haplomitosis, Cryptohaplomitosis, Eurypanmitosis, Cyclomitosis or Polymitosis, Polyrheomitosis, Metamitosis, etc.

their possible relations to one another or to any process of evolution. The apparent object of the complex mechanism of a mitotic figure is to ensure the exact bipartition of the hereditary complex represented by the chromosomes. These elements, and the chromatin of which they are composed, are the most important, while the kinetic elements with which they are associated in division, as agents in the process, are of secondary importance. I shall consider first, therefore the chromatin of protozoan nuclei and its history during nuclear division.

(*a*) **Chromatin and Chromosomes.**—The conception of chromosomes, as they appear in Metazoa, is definite and consistent throughout. They are formed at certain periods of cell activity (prophase of division) by the aggregation of chromomeres into nuclear bodies of definite form and size, and the number is constant for all somatic and germ cells in the same species. Each chromosome is specific and retains its individuality from generation to generation by cell division. At the end of division it resolves itself into an aggregate of chromomeres which, in some cases, are found to be confined to a definite part of the nucleus (chromosomal vesicle) at the prophase of the following division these same chromomeres re-collect to form the chromosome which divides into equal parts by longitudinal division. The chromosomes, furthermore, are qualitatively different, no two of them being identical. At one meiotic division, finally, the number of chromosomes is reduced to one-half by the separation of half of them from the other half, thus resulting in two types of nuclei which are entirely different in chromosomal make-up.

An analysis of the literature dealing with the so-called chromosomes of Protozoa shows that there has been little or no consistent use of the term. To many observers the word is used to describe any chromatin which happens to be in the center of a division figure and without regard to other conditions which limit and define the chromosome as a definite thing, viz.: A definite number in the cell, longitudinal division, qualitative differences, reduction in number at maturation, etc. It is true that in only a few cases among the Metazoa has it been demonstrated that chromosomes have a specific individuality combined with qualitative differences, but the striking similarity in dividing chromosomes of all Metazoa and the same complicated mechanism in all cases for their equal distribution to daughter cells, give a basis upon which the generalization rests. We have no basis, however, for extending the generalization to Protozoa, for here we have absolutely no evidence of qualitative differences and no evidence of individuality. In some cases we have evidence that structures in the center of a division figure are formed by the fusion of chromomeres, and some evidence that such structures divide longitudinally. These two conditions, which

are relatively rare, are the only conditions whereby many of the so-called chromosomes of Protozoa resemble those of Metazoa, and if we use the term chromosome at all it should be in a definite, limited, morphological sense and only for those nuclear structures of Protozoa which conform in origin and in fate to chromosomes of Metazoa. I shall use the term chromosome, therefore, only for those compact intranuclear aggregates of chromomeres which divide as unit structures and which are resolved into chromomeres after such division.

A brief review of some of the frequently recurring types of chromatin structure at the time of nuclear division will show how difficult it is to speak with assurance of chromosomes in Protozoa. The series is not to be construed as an effort to establish a phylogenetic chain of stages culminating in well-defined chromosomes, nor as a means of pointing out that one is a "higher" type than another. Certain vital functions are undoubtedly associated with the nucleus and with the chromatin of the nucleus, and the fact that some types of organisms with peculiar nuclei continue to live and reproduce is evidence enough that such nuclei are adequate for their needs. The variations in type arise through the association of chromatin with other nuclear or cytoplasmic constituents, and this involves more or less formality in preparation for its perpetuation by exact bipartition to daughter cells. All traces of chromosome formality, however, as well as reduction processes, appear to be absent in gamete nuclei formed by rhizopod chromidia.

One group of types is represented by massive nuclei as found in the macronuclei of the Infusoria. Here the resting nuclei are made up of closely packed granules or chromomeres and there is no formality nor mechanism associated with their division during reproduction. Each granule elongates and divides into two parts, thus doubling the number of chromomeres. The mass thus formed is passively distributed to the daughter cells by division of the nucleus through the center. It is a quantitative distribution, for the daughter nuclei do not contain representative halves of the individual chromomeres and the inference is that all of the chromomeres are qualitatively identical. To this type also I would assign the peculiar chromatin granules of *Dileptus anser* which are distributed throughout the protoplasm unconfined by a nuclear membrane. Each granule divides where it happens to be and with the majority of granules both halves remain in one daughter cell after division (Fig. 58).

In another group of types we have to do with vesicular, endosome-containing nuclei. The endosome may or may not contain an endobasal body. It is well represented by the nucleus of *Spongomonas splendida* according to the observations of Hartmann and Chagas (Fig. 59). Here, according to the description, the mass of chromatin

of the resting nucleus divides into two equal masses without fragmentation at any stage. Similar conditions are shown by the gregarine *Gonospora varia* according to Brasil (1905), by *Amœba diploidea* according to Hartmann and Naegler (1908) and by the simpler amœbæ (Fig. 61).

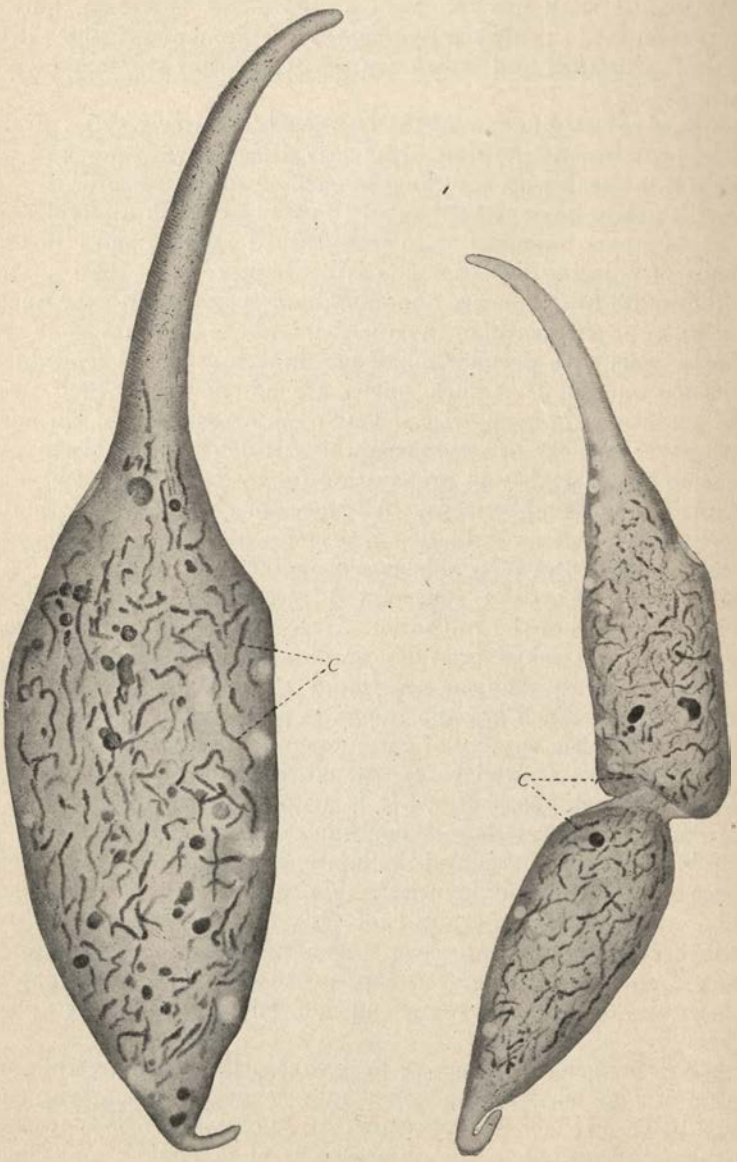

FIG. 58.—Division of *Dileptus anser*. The elongated chromatin granules (*C*) divide where they happen to lie. (Original.)

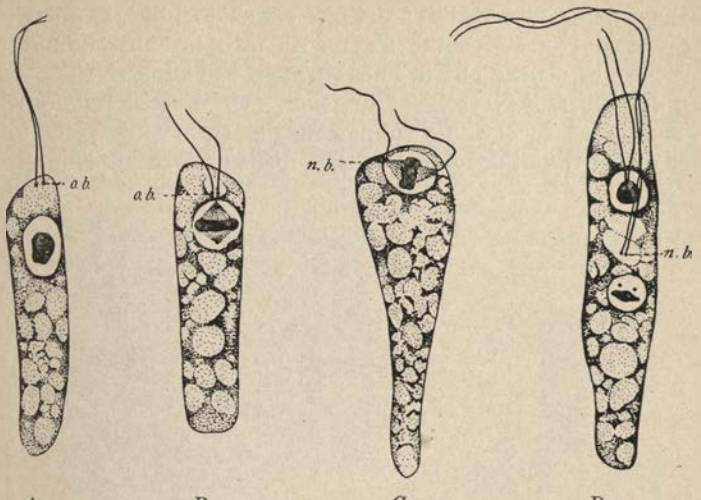

FIG. 59.—Division of *Spongomonas splendida* Hart. and Ch. The old flagella are discarded and new ones form from the centrioles (*C* and *D*). (*o.b.*) old blepharoplasts; (*n.b.*) new blepharoplasts. (After Hartmann and Chagas.)

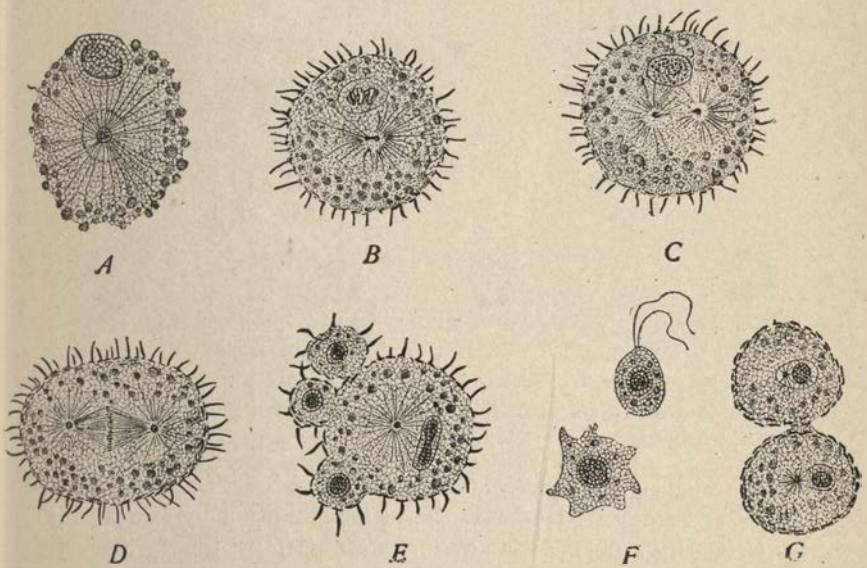

FIG. 60.—Nuclear division and budding in Heliozoa. *A*, Vegetative cell of *Sphærastrum* with axial filaments focussed in a central granule (centroblepharoplast); *B*, *C*, *D*, division of central granule and spindle formation in *Acanthocystic aculeata*, *E*, *F*, formation of buds of same; *G*, exit of central granule from the nucleus of young cells. (After Schaudinn.)

In another group of types the chromatin of the resting vesicular nucleus is contained also in a definite endosome, but, in preparation for division, the endosome fragments into minute chromomeres, which may be strung out in lines through the nucleus, these strings being divided transversely at division. Or the chromomeres may be aggregated in a fairly homogeneous transverse plate in the center of the dividing nucleus. The former condition is illustrated

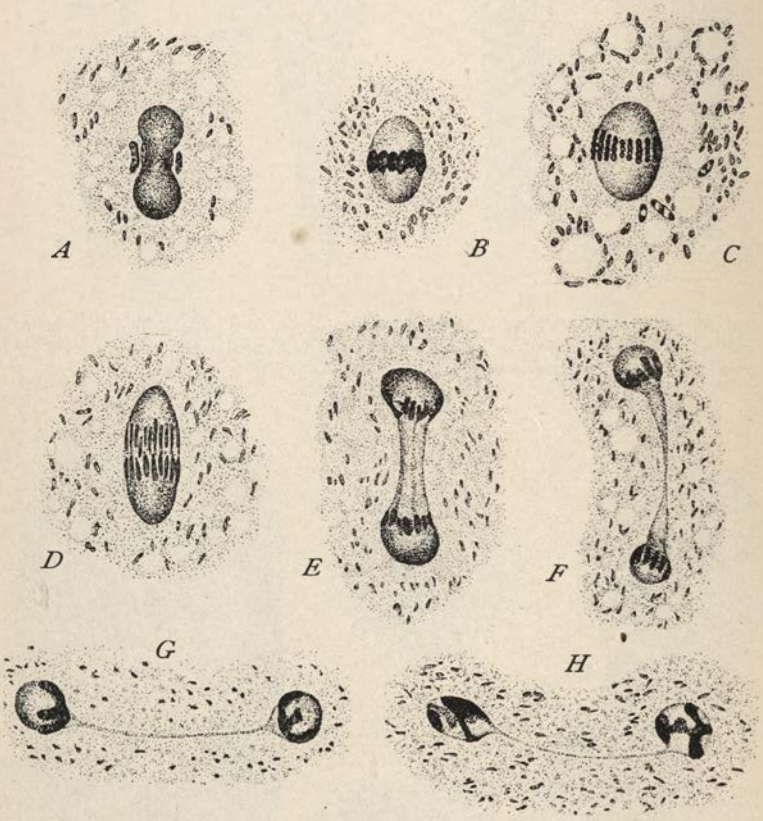

FIG. 61.—Successive stages in the nuclear division of a simple *Amœba*. (After Wasielewsky and Kühn.)

by the nucleus during vegetative division of *Actinosphærium eichhornii* according to Hertwig, the latter condition by *Acanthocystis aculeata* (Fig. 60), *Paramœba chætognathi*, or the myxomycete *Comatricha obtusata* according to Lister.

A slight modification of this type is shown by nuclei containing multiple endosomes as in *Pelomyxa binucleata* which fragment at periods of division, giving rise to a granular nuclear plate (?) which

presumably divides to form the daughter plates as shown in Schaudinn's well-known figure, or to division figures like that of *Centropyxis aculeata*.

Another widely distributed type of division figure is derived from vesicular nuclei in which the chromatin is not contained in one or more endosomes but is distributed peripherally about the nucleus where it usually forms a distinct chromatin reticulum. Such nuclei usually contain an endosome which may be the most conspicuous structure of the nucleus. In *Amœba crystalligera* the peripheral chromatin appears to be passively divided without any appreciable change in its make-up. In *Amœba vespertilio* the peripheral chro-

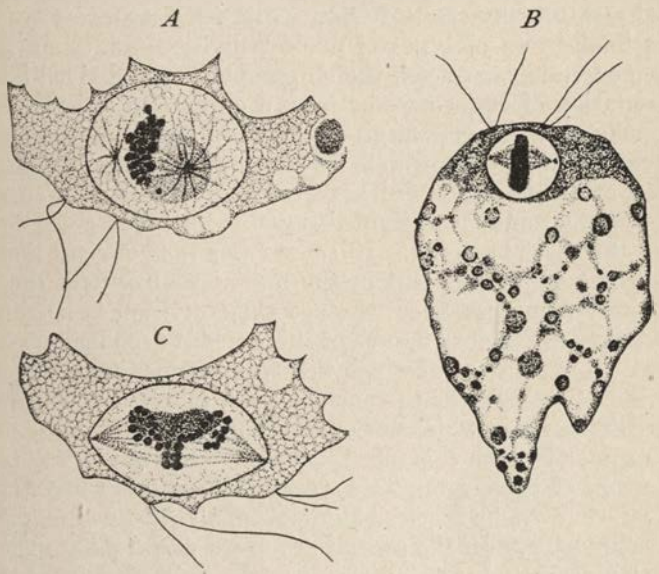

FIG. 62.—Nuclear division in *Collodictyum triciliatum*. (After Belar.)

matin is similarly divided and distributed but the endosome apparently contains some chromatin in addition for a complete division figure is formed from its substances, chromatin-like granules forming a nuclear plate (Fig. 39, p. 82). In other cases, as for example *Endamœba intestinalis* and *E. cobayæ*, the peripheral chromatin is broken up into chromomeres, which collect in the center of a spindle from the linin of the nucleus and with centrioles at the poles (Fig. 26, p. 63).

In still another general type, derived also from vesicular nuclei, the chromatin in the form of chromomeres is suspended in a loose reticulum. In *Opalina* they appear to be aggregated in a few larger granules, which divide where they happen to be without further

formality, the nucleus meantime assuming an indefinite division figure. More frequently, however, the chromomeres are suspended between an endosome and the nuclear membrane, as in *Thylacomonas compressa*, *Eutreptia viridis*, *Eimeria schubergi*, *Oxyrrhis marina*, or various species of *Trypanosoma*. In some of these, at division the chromomeres appear to form a nuclear plate, and are distributed in equal groups to the daughter nuclei (Fig. 62). In *Euglena viridis*, which usually is placed in this group, the chromomeres according to Tschenzoff (1916) are derived from a chromatin reticulum and pass through a skein stage before forming a broad nuclear plate in which each is longitudinally divided. In *Oxyrrhis marina* the chromomeres unite to form linear aggregates which divide longitudinally (Hall, 1925). (Fig. 43, p. 88).

In a final group of types of nuclear division figures either from massive or vesicular nuclei, the chromomeres are derived from the fragmentation of endosomes or from a chromatin reticulum. The common feature in this large group is the fact that these chromomeres unite secondarily to form definite chromatin bodies which satisfy, in part at least, the definition of chromosome as given above. These chromosomes are divided equally, one-half going to each pole of the division figure. In some cases it is obvious that their division is longitudinal, but in the majority of cases it cannot be ascertained with assurance whether their division is longitudinal, or transverse. Nuclear figures of this general type may be divided into two groups, in one of which the chromosomes are too numerous to permit of decision as to their constant number, and the second comprising forms in which the chromosomes are constant in number and in some of which this number is reduced to one-half at meiosis. In the first of these groups we would include types like *Euglypha alveolata*, the various species of *Paramecium*, *Noctiluca miliaris* and *Oxyrrhis marina*. In the second group we would place such forms as *Actinophrys sol*, *Aggregata eberthi*, *Trichomonas* and allied flagellates, *Trichonympha* and related forms, and the majority of ciliates in which the maturation processes are known.

In *Euglypha alveolata* the chromatin of the vesicular nucleus is distributed throughout the resting nucleus. During the early division stages the chromomeres are rearranged in rods or fibrils which form a more or less definite skein within the nucleus; this skein fragments into a large number of chromosomes which are longitudinally divided according to Schewiakoff. A more aberrant history is followed by the chromatin of the nuclei of various species of *Paramecium*. In *Paramecium caudatum* the micronucleus belongs to the massive type, and there is no satisfactory account of the origin of chromosomes in vegetative division. The micronucleus becomes much larger, however, in preparation for the first maturation division, when, according to Calkins and Cull (1907), the com-

pact mass of chromatin spins out into an elongated reticulum from which about 150 double chromosomes are formed by transverse segmentation. These are divided longitudinally during the transition from the characteristic "crescent" to the full spindle figure (Fig. 206, p. 496). Dehorne (1920) on the other hand maintains that only one long, convoluted skein of chromatin is formed and divided as such, therefore no chromosomes at all are in *Paramecium*.

The division figure of *Noctiluca miliaris* is quite different from the others of this group. The chromatin, according to observations of Ischikawa and of Calkins (1895), is contained in a number of large endosomes (chromatin reservoirs), each of which breaks up into a mass of chromomeres. These collect in chromosome strings, ("chromospires") which are oriented toward one pole of the nucleus and are far too numerous to count. After division of the centrosphere, the nucleus elongates and bends around the connecting centrodesmose in such a manner that the chromosomes form an incomplete annular nuclear plate between which and the centrodesmose, the nuclear membrane is absorbed. Mantle fibers, attached to the ends of the chromosomes, focus in a centriole in each daughter sphere, and with the separation of the daughter centers, each chromosome is longitudinally divided (Fig. 52, p. 101).

A more definite Metazoön type of chromosome formation is shown by the organisms comprising the second group above. Here the number of chromosomes is usually smaller and their individual history during nuclear division is less difficult to make out. A good example, typical of the plymastigote flagellates, is *Trichonympha campanella*, as described by Kofoid and Swezy. Here the resting nucleus contains a large granular endosome. In the prophase of division the granules of this endosome give off chromatin along the walls of the linin reticulum until a definite skein stage results (Fig. 51, p. 100). Double chromosomes, 26 in number, and formed by the splitting of the spireme segments, make up a definite nuclear plate. They are attached by intranuclear fibers to the daughter blepharoplasts and are divided longitudinally with the division of the nucleus. The original connecting fibrils between the separating halves of the blepharoplast ("centroblepharoplast") remain at all times outside the nuclear membrane, hence it is called a paradesmose by Kofoid and Swezy (see *Noctiluca*). One of the chromosomes appears to be different from the others, both in resting and division stages, and is called the heterochromosome, although its function or significance is quite unknown. Similar odd chromosomes are known in some Gregarinidæ and Coccidiida where the vegetative stages are haploid, as well as in other polymastigote flagellates. Except for the complications brought in by the extensive neuromotor apparatus of *Trichonympha campanella*, the division figures of other, related, flagellates are quite

similar, although the number of chromosomes is usually smaller. Thus Kofoid and his collaborators found about 24 in *Leidyopsis sphærica*, 12 in *Trichomitus termitidis*, and 4 in *Giardia muris* (Fig. 140, p. 293).

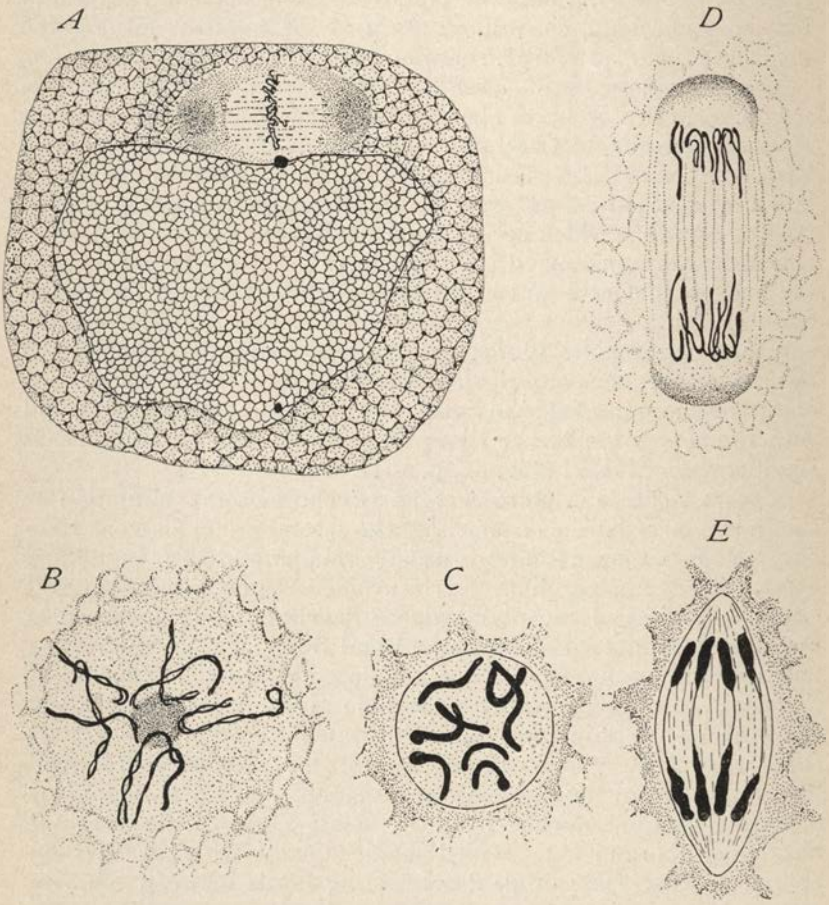

FIG. 63.—*Monocystis rostrata* chromosome reduction. *A*, Formation of spindle in pseudo-conjugant; *B*, *C*, nuclear plates of progamous divisions, 8 chromosomes; *D*, anaphase of same; *E*, anaphase of last progamous division, the number of chromosomes is here reduced from 8 to 4. (After Mulsow.)

A small number of chromosomes is likewise found in a number of the Gregarinida, and their history in division approaches that of metazoan chromosomes. Thus in the case of *Monocystis rostrata* Mulsow describes 8 definite chromosomes formed from a portion of the nuclear chromatin, the number being reduced to 4 in the gamete-forming divisions (Fig. 63). Shellack and Léger, also, have described

similar chromosomes in *Monocystis ovata* and in *Stylorhynchus longicollis*. In the latter case, also, there is a peculiar lagging heterochromosome ("axial chromosome") of unknown significance.

Finally, in the maturation divisions of many ciliates, a small number of chromosomes, and the reduction to half the normal number, have been described by several different observers. Two fairly definite types of chromosome formation occur, according as the resting nucleus is vesicular or massive in structure. The majority of massive micronuclei behave more or less like the micronucleus of *Paramecium caudatum*, forming a crescent or some other equally characteristic figure during the prophase of nuclear division. Thus in *Chilodon uncinatus* according to Maupas and later Enriques, the chromatin is drawn out first in the form of an elongate cross-shaped band, while in *Vorticella monilata* and in *V. nebulifera*, according to Maupas, and in *Opercularia coarctata*, according to Enriques, a similar chromatin rod extends the entire length of the cell. The characteristic type of nuclear figure formed by the resting vesicular nuclei is represented by *Onychodromus grandis* (Maupas), *Bursaria truncatella* (Prowazek), *Didinium nasutum* (Prandtl), *Anoplophrya branchiarum* (Collin), *A. circulans* (Brumpt, 1913), and *Uroleptus mobilis* (Calkins). In all of these cases the two poles of the spindle are not formed simultaneously. The chromatin granules into which the compact chromatin mass fragments are retained at one end of the micronucleus, where they form the chromosomes. The first pole of the later spindle is formed by the migration (in *Uroleptus mobilis*) of a centriole from this aggregate of chromomeres to the opposite pole of the nucleus (Fig. 36, p. 78). After the chromosomes are established the second pole is formed by the migration of the remaining centriole to the opposite part of the nucleus. The chromosomes in all cases are compact, granular aggregates of chromomeres, and, being spheroidal, afford no evidence of either longitudinal or transverse division. Their number, in many cases, is sufficiently small to permit of exact counting; 20 (?) in *Ophrydium versatile* (Kaltenbach); 16 in *Carchesium polypinum* (Popoff), *Chilodon uncinatus* (Enriques), *Didinium nasutum* (Prandtl) and *Opercularia coarctata* (Enriques); 12 (?) in *Bursaria truncatella* (Prowazek); 8 in *Uroleptus mobilis* (Calkins); 6 in *Anoplophrya branchiarum* (Collin) and in *Stylonychia pustulata* Prowazek); and 4 in *Boveria subcylindrica* (Stevens). In the majority of cases where reduction in number to one-half has been made out, viz.: in *Carchesium*, *Chilodon*, *Didinium*, *Opercularia*, *Uroleptus*, and in *Anoplophrya*, the reduction in number of chromosomes occurs with the second meiotic division. Between the two maturation divisions the micronuclei rarely return to the massive structure characteristic of the resting micronuclei. The phenomenon of synapsis or pseudo-synapsis is represented in a large

number of Protozoa by somewhat peculiar relations of the chromosomes during both vegetative and maturation divisions of the nucleus. It results in the formation of double chromosomes which appear to be longitudinally split. Thus in *Uroleptus mobilis* the 8 chromosomes of the first maturation division unite in 4 pairs which are split at the second maturation division, the resulting nuclei having 4 single chromosomes (Fig. 218, p. 525). Here is undoubted

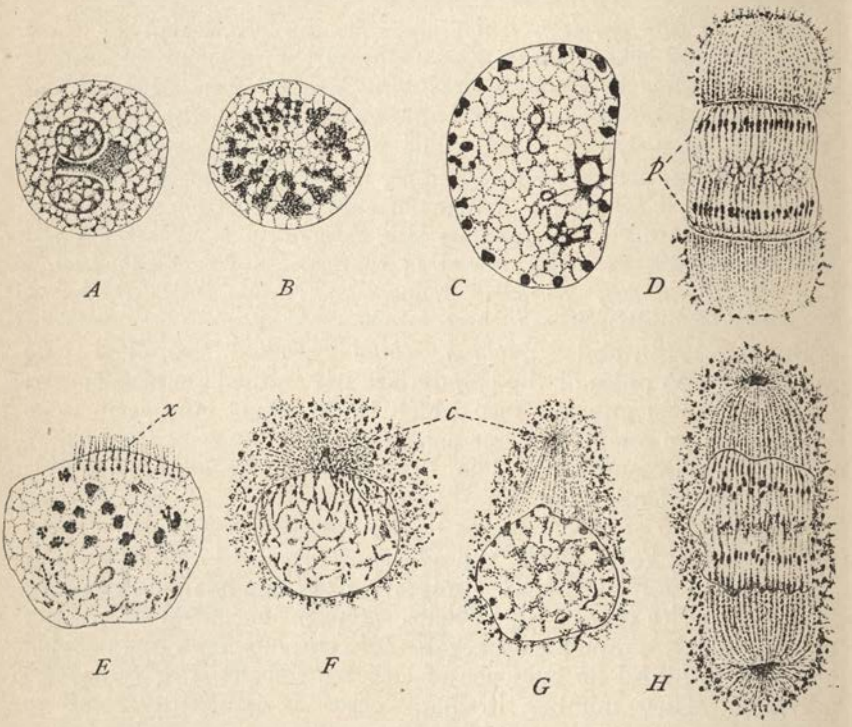

FIG. 64.—*Actinosphærium eichhornii* origin of centrosome from nucleus. (After Hertwig.)

synapsis and reduction. But in *Boveria subcylindrica*, Stevens describes the similar union of the 4 chromosomes to form 2 during the vegetative divisions and analogous conditions are evidently found in the polymastigote flagellates according to the observations of Kofoid and his collaborators. The significance of this apparent reduction in vegetative mitosis is problematical, and the subject requires further careful study (see Chapter XI for details of meiosis.)

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CHAPTER III.

STRUCTURAL DIFFERENTIATIONS.

Although fundamentally important in vital functions, the various granules and structures which have been described can hardly be regarded as obvious or visible characteristics of Protozoa. Careful study, involving elaborate technical methods, is necessary to reveal the parts they play, and for some, at least, even this has not yet yielded positive results.

The visible characteristics, those we see upon casual examination with a microscope—form, color movement, shells, tests, stalks, etc. —are secondary in importance in respect to the ultimate vital activities. It is in connection with these, however, that the Protozoa are best known and the peculiar fascination which they have for the microscopist is mainly due to these obvious features. The outer structures which please the eye, or the motile organoids which cause the fascinating endless variety of movements, represent the outcome or product of the ceaseless activities going on between the various constituent elements of the protoplasm. Some of them are necessary for the continued life of the organism, some are useful in one way or another, but not absolutely necessary, and some, *e. g.*, the scalloped cuirass of *Entodinium*, have no obvious reason for being.

In some types of Protozoa, even on superficial examination, it is evident that the aggregate of substances making up the protoplasm is differentiated into an external zone and an internal, medullary part. The external portion is usually called ectoplasm, the inner part endoplasm. The ectoplasm is that part of the protoplasm which comes in direct contact with the environment. It is the part through which food substances must pass into the organism and through which the waste matters of destructive metabolism, as well as undigested food, must be voided to the outside; it is the part which first receives external stimuli of various kinds, and it is the part which gives rise to the more easily visible portions of the locomotor structures, and to the specializations for support and protection.

Acting thus as a medium of exchange between the living protoplasm and the external world, the ectoplasm has become modified in ways that would be impossible for the endoplasm. In simple cases, as, for example, in *Amœba proteus*, it is not strikingly different from the endoplasm, but in other cases it becomes a complex of

specialized adaptations and the source of many important organoids of the cell. Here it is quite different from the inner protoplasm in structure and in function and the aggregate, to distinguish it from the relatively simple ectoplasm of *Amœba* is better known as the cortical plasm, or simply the *cortex*.

1. DIFFERENTIATIONS OF THE CORTEX.

It is quite probable that there is no such thing as an entirely naked cell among the Protozoa. Even in *Amœba proteus*, the classical example of a naked cell, the ectoplasm is covered by a delicate, viscous hyaline zone of modified protoplasm. Hofer, Verworn, and others, have noted it in connection with food taking; Schaeffer (1917), in connection with movement claiming that it is a third kind of protoplasm in addition to ectoplasm and endoplasm and Chambers (1915) came across it in connection with microdissection experiments. Among Sporozoa and Infusoria it has been described in many species, and in flagellates and ciliates it is not infrequently characterized by definite markings or sculpturing. It is the most external portion of the cell and is distinguished from the remainder of the cortex by the special name *periplast* or *pellicle*.

The periplast always fits the body closely, dividing when the body divides, thus differing from all other types of lifeless coverings; in *Paramecium caudatum*, for example, during plasmolysis, it becomes separated from the rest of the cortex and distended by the accumulation of fluids. In other cases it is much more definite and membrane-like as in *Cochliopodium bilimbosum* (Fig. 8, p. 30), or in the loricate ciliates such as *Euplotes harpa*, *Uronychia setigera* and their allies. Periplasts are frequently delicate enough to give way to forces generated within the body, but elastic enough not to break, a phenomenon resulting in peristaltic movement which is not infrequent in Gregarinida (*e. g.*, *Monocystis agilis*) and in flagellates (Euglenida). Such organisms are said to be "metabolic" and the peculiar motion is sometimes called "euglenoid movement."

In many cases the periplast is ornamented by striations which usually run obliquely down the cell (*Phacus longicaudus*, *Euglena oxyuris*, Fig. 65); in some cases by ridges (*Phacus pyrum*, *Chloropeltis sp. Menoidium incurvum*, etc., Fig. 65, *D*, *F*); by furrows or by nodules as in the ciliate *Vorticella monilata*. In *Coleps hirtus* the periplast is differentiated into definite plates of characteristic form arranged in four girdles which compose an armature for the organism (Fig. 65, *A*, *C*). The skeletal structures of *Diplodinium ecaudatus* are likewise differentiations of the periplast (p. 20).

Not only the periplast, but the entire cortex has become differentiated in a great variety of ways in response, apparently, to the many demands made upon it as a result of its contact with the

environment. These may be grouped as cortical differentiations for (*a*) support and protection; (*b*) locomotion and irritability; and (*c*) food-getting and defecation.

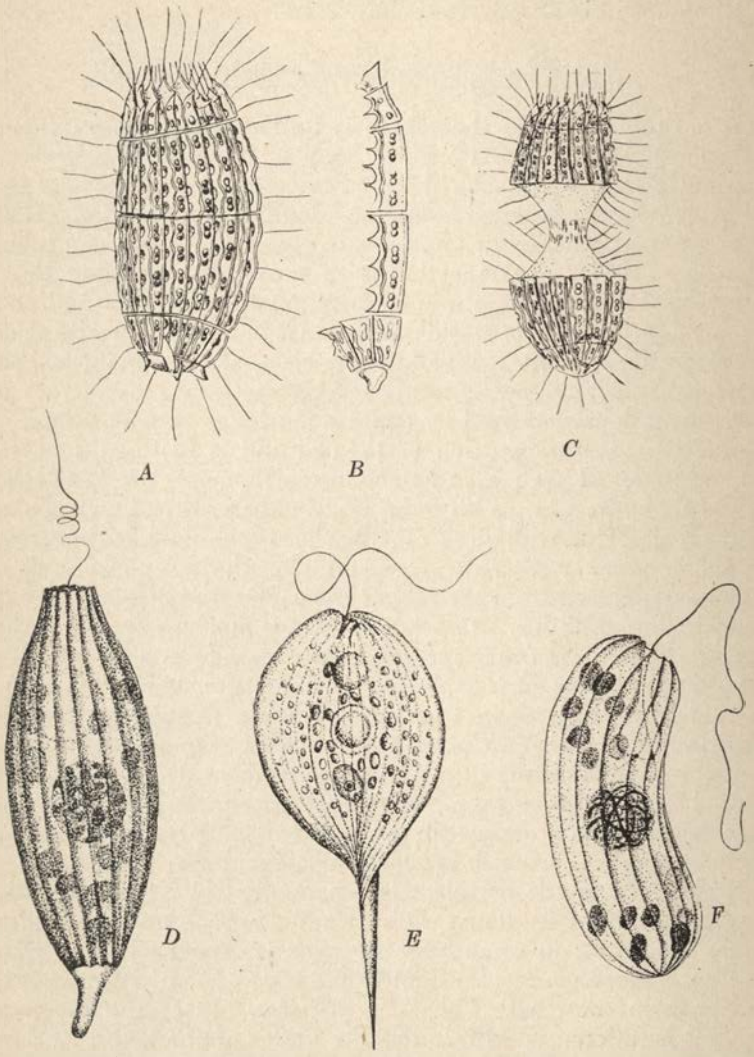

FIG. 65.—*A*, *B*, *C*, Form, structure of plates, and division of *Coleps hirtus* (after Maupas); *D*, *Chloropeltis* sp. (Original); *E*, *Phacus longicaudus* (after Stein); *F*, *Menoidium incurvum* (after Hall.)

(*a*) **Cortical Differentiations for Support and Protection.**—Apart from the thickening and hardening of the periplast which furnishes sufficient protection and support for the great majority of flagellates

and ciliates, the cortex is the seat of precipitation of different mineral substances; of secretion of gelatinous substances; or of protoplasmic modifications into lifeless organic substances of various kinds. These various products of cortical activity are moulded into close-fitting, lifeless membranes of chitin, pseudochitin, and cellulose, or into loosely-fitting shells, tests, skeletons, cups, tubes and the like. These are not divided when the cell divides but are either left as empty shells and tests, or one of the daughter individuals after reproduction remains in the old shell while the other individual makes a new shell for itself.

Gelatinous mantles are common in flagellates and are occasionally found in the ciliates (*e. g.*, *Ophrydium versatile*), but gelatinous materials are secreted by all types of Protozoa. Usually, when the secretion is abundant, daughter cells remain embedded in it as a matrix after division, and the so-called spheroidal types of colony result (see p. 34).

The most characteristic shell-forming material manufactured by Protozoa is chitin and pseudochitin. Chemically chitin is a modified protein ($C_{30}H_{50}O_{10}N_4$ or multiple) and undoubtedly polymorphi in composition. Its mode of formation is still uncertain but conditions in Protozoa support the view of Chatin that it arises by transformation or differentiation of the peripheral cellular protoplasm. Not only are cups, tests, "houses" of various kinds formed of these substances, but cyst membranes, spore capsules of the Sporozoa and "central capsules" of the Radiolaria as well, while impregnated with calcium carbonate, silica, strontium sulphate, etc., or covered by foreign bodies of different kinds, the chitinoid membranes furnish the framework for the up-building of the most complex shells and skeletons. In encysting ciliates the animal becomes spherical, and much condensed and is surrounded by an envelope of fluid-like material which condenses more and more with exposure until the definite membrane, impervious to moisture and resistant to all unfavorable conditions of the environment, results. In Radiolaria the central capsule is a spherical wall of chitin, separating the endoplasm from the external protoplasm and perforated in various ways to permit of communication between the different regions of the cell (see p. 343).

In flagellates and ciliates the chitinous houses, tests, cups, etc., are usually colorless and very transparent, but in the rhizopods this is unusual, the chitin shells being colored by oxides of iron usually red or brown (*Arcella sp.* etc.). In the majority of fresh water rhizopods the outer surface of the chitinoid shell is covered by foreign particles of various kinds, such as sand crystals, diatom shells, or even living algæ which are glued to the membranes by a chitinous cement. Similar shells, which are generally known as arenaceous shells, are found amongst the Foraminifera. In other cases, plates

of silica are deposited in the inner protoplasm and passed out during reproduction to be cemented on the chitinous membrane in regular patterns (*Euglypha alveolata*, Fig. 8, p. 30). Foreign bodies caught up in the wrinkles of withdrawing pseudopodia are similarly stored in the protoplasm to be used for shell-building purposes, Verworn, for example, compelling *Difflugia* to build its shell of different kinds of powdered glass.

The lime shells of Foraminifera are formed in quite a different manner. Here, calcium carbonate is precipitated between two lamellæ of chitin very much as a cement wall is made between board surfaces. Except for a single mouth opening such limestone shells may form an unbroken wall about the organism (imperforata) or

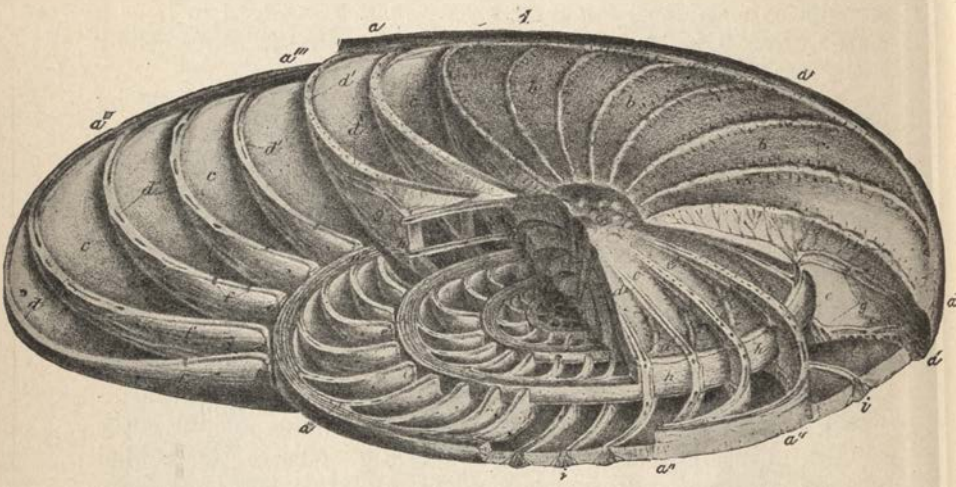

FIG. 66.—A complex polythalamous shell of *Operculina* (schematic). The shell is represented as cut in different planes to show the distribution of the canals and the arrangement of septa and chambers. (After Carpenter.)

they may be perforated by myriads of minute pores (foramina) through which the pseudopodia pass to the outside, a condition which gave rise to the name Foraminifera. In the more complicated types of these limestone shells, which may reach a diameter of 2 or 3 inches, the calcium carbonate may be deposited at successive intervals of growth, thus giving rise to chambered structure of the cells. Such polythalamous shells are complicated by the presence of an intricate system of canals which, in life, are filled by moving protoplasm (Fig. 66).

Skeletons of Heliozoa and Radiolaria, unlike the more clumsy shells of the Foraminifera, are usually delicate in structure and graceful in design. They are formed for the most part by a deposit of silica upon a chitinous base. Dreyer has given evidence to indicate that

such skeletons have their beginnings in spicules which conform in shape and size with the nodal points in the alveolar walls of the cytoplasmic reticulum (Fig. 11, p. 33). Isolated spicules are characteristic of several Heliozoa and Radiolaria where they form a loose or felted covering in the outer protoplasm. Such spicules invariably grow by accretion, that is, by the addition of new substance to the outside of that already formed. If such added material is formed in a limited region of the protoplasm, the result is a continued accretion of silica to the end of a spicule which is pushed

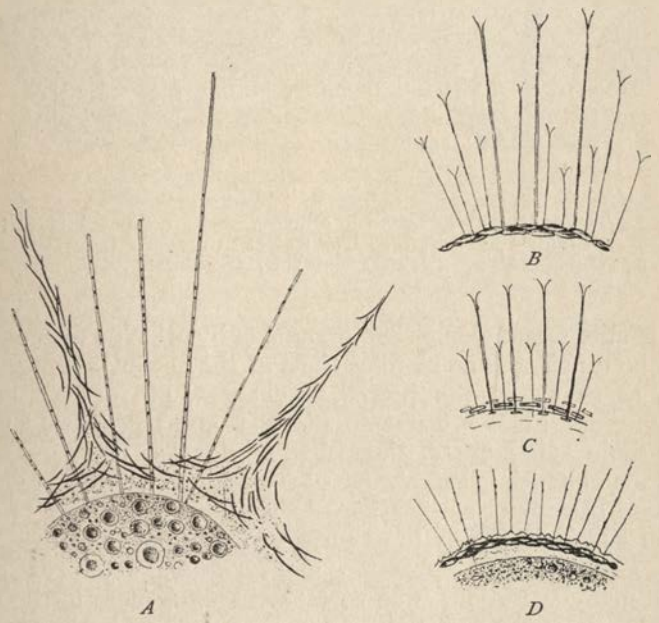

FIG. 67.—Types of spicules in Heliozoa. *A*, *Raphidiophrys pallida* with curved silicious spicules; *B*, *Pinaciophora rubiconda* with tangential plates and forked spines; *C*, *Acanthocystis turfacea*, with separated plates and forked spines; *D*, *Pinaciophora fluviatilis*. (From Calkins after Penard.)

farther out with each increment, thus giving rise to long bars and spines which are radially arranged in forms like *Acanthocystis aculeata*, etc. (Fig. 67). The silicious deposit, again, may be made throughout a zone completely surrounding the center, resulting in clathrate or latticed skeletons of varying grades of complexity (*Clathrulina elegans*, *Nassellaria*).

Cellulose mantles are limited almost entirely to forms provided with chlorophyll, and able to form starch by virtue of the energy of the sun's rays. Cellulose is a by-product of these cells and is secreted either as a uniform covering for the entire organism, as in many

Chrysoflagellida and Chloroflagellida (Fig. 125, p. 258), or, as in the Dinoflagellida, it is deposited in the form of definite plates, which may be highly sculptured or drawn out into characteristic ridges, ledges or spines (Fig. 68).

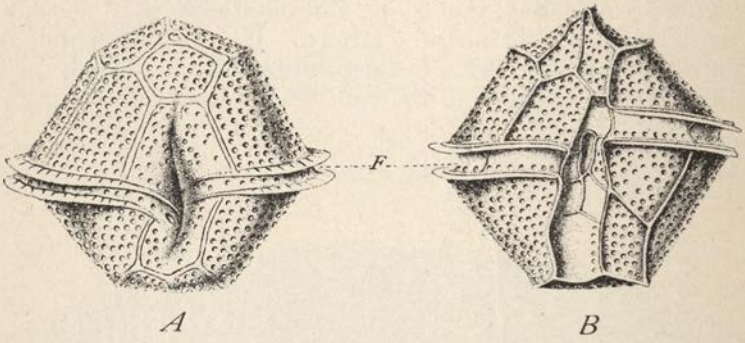

FIG. 68.—Types of Peridinidæ with cellulose shells composed of plates. *A*, *Goniodoma acuminatum* Ehr.; *B*, *Gonyaulax* Kof. (*F*) transverse furrow with epitheca above and hypotheca below. (*A*, after Schütt; *B*, after Kofoid.)

(*b*) **Motile Organoids.**—The organoids by which Protozoa move are to be considered as modifications of the cortex, although some types, as shown in the preceding chapter, are derived in part from internal kinetic elements (flagella and some pseudopodia). Three main types are distinguishable—flagella, pseudopodia and cilia, each of which is sufficiently distinct from the others to furnish a natural basis for classification of the Protozoa, a basis of classification which Dujardin first employed to create the three great groups *les flagellés*, *les rhizopodes*, and *les ciliés*. Each type is subject to many variations, due to inherent differences in the motile organoids themselves, or to fusion in various ways leading to structures of considerable complexity.

It is extremely difficult to decide whether flagella or pseudopodia are the more primitive in type. From most general text-books on Zoölogy we learn that the matter admits of no question, and are taught that the pseudopodium is the most primitive form of motile organ in the animal kingdom. This certainly has been the most widely accepted view. Many a generalization referring to Protozoa, however, which has found its way into general works on Biology, appears to have been drawn from the conditions in some one organism which is conspicuous by reason of its abundance and ease of study. It would sometimes appear, indeed, that the common species of *Paramecium* and *Amœba proteus*, to many general writers, constitute the Protozoa. This seems to be the case with the problem of pseudopodia and flagella, the argument being that a pseudopo-

dium of *Amœba proteus* is certainly a less complex motile organ than the flagellum of *Euglena viridis*, and therefore more primitive. Had the comparison been made between the pseudopodia of *Actinophrys sol* or *Acanthocystis aculeata* and a typical flagellum, the conclusion would not have been so obvious. There is a good deal of evidence against the generalization as it is usually expressed. In the first place, a pseudopodium of *Amœba proteus* cannot be interpreted as a motile organ. It is not a definite structure in the cell, not does it cause the body of *Amœba proteus* to move. On the contrary, it exists because of the movement of the body protoplasm and the pseudopodium is merely the visible, physical expression of this movement which, in turn, is due to the transformation of energy in destructive metabolism. This energy finds its vent in that portion of the ectoplasm which, for the time being offers the least resistance; the ectoplasm gives way at this point, the endoplasm gushes through and a pseudopodium results (see Chapter IV, p. 172). Such pseudopodia are not the source of movements of the cell, they are results, not causes, of movement. The pseudopodia of Heliozoa, on the other hand, are motile organs, and the axial filaments which they contain are regarded as equivalent in structure and in mode of origin to the kinetic elements of flagella. The pseudopodia of Foraminifera are intermediate between those of Heliozoa and those of testate rhizopods. The problem, then, comes down to a theoretical question of probabilities. Is it more probable that pseudopodia of the type found in *Amœba proteus* become progressively differentiated into motile organs through stages like the finger-formed pseudopodia of the testate rhizopods, the reticulate pseudopodia of Foraminifera and axopodia of Heliozoa and Radiolaria, to the typical motile organ of the flagellate type? Or is it more probable that a motile organ originating from a definite kinetic center (basal body or blepharoplast) has become progressively indefinite with loss of the kinetic elements through the same series of forms, but in the opposite direction, and ending in types like *Amœba proteus?* To my mind, the pseudopodia of *Amœba proteus* and its immediate relations, have no place at all in such a series; they are merely expressions of the physical conditions of the protoplasm and of the forces operating within, and they may appear in any cell having an appropriate physical make-up. Thus we find them in certain types of cell (leukocytes and phagocytes) widely distributed throughout the animal kingdom, and we find them here and there, in every group of the Protozoa.

An illuminating illustration in support of this conclusion is afforded by the transitory flagellated stages of one group of amœboid organisms, the Bistadiidæ (see p. 337). Here, in *Nägleria gruberi*, for example, the organism loses its pseudopodia under certain conditions, and develops flagella, not by metamorphosis of the

pseudopodia, but from blepharoplasts which, as centrioles, emerge from the nucleus (Fig. 42, p. 86).

For these reasons I believe that the flagellum type of motile organs is the most primitive type we know while axopodia and myxopodia, the former with kinetic elements of weakened function, the latter with denser axial protoplasm which Doflein also interprets as equivalent to axial filaments, represent stages in the deterioration of the kinetic function coincident with the absence of definite kinetic centers (see also p. 141). For these reasons also, together with others which will be given later, we hold with Doflein (1916), Klebs and many others, that the group of flagellates furnishes more evidence of original ancestry than do the rhizopods (see p. 251).

1. **Flagella.**—Flagella are widely distributed throughout the animal and plant kingdoms, forming the motile elements of animal spermatozoa and of plant zoöspores, or current-producing organs of the collared cells of sponges. They are sometimes combined with pseudopodia (*Dimorpha mutans*, Fig. 12, p. 34, *Mastigamœba invertens*, Fig. 137, p. 287, *Ciliophrys infusionum*, etc.), sometimes with cilia (*Myriaphrys paradoxa*, Fig. 160, p. 369).

Flagella are usually excessively fine and delicate fibers extremely difficult to see and to study in the living organism. In the great majority of cases the finer structure has not been made out, but in a few favorable types some progress has been made. In these cases it is known that the flagellum is made up of two definite elements, an axial, highly vibratile filament, which is formed as an outgrowth from the basal body or blepharoplast, and an enveloping elastic sheath which is formed from the protoplasmic substance of the cortex. In some cases the sheath is circular in cross-section (see Plenge), in others ellipsoidal, while the contractile thread which is usually attached firmly to the sheath, may run in a straight line the entire length of the sheath, or may follow a spiral course. In the majority of flagellates the sheath undulates and vibrates in unison with the contractile axial thread, but in a few types, such as *Peranema trichophora* or certain species of *Astasia*, the sheath remains passive while the axial thread extends freely beyond the limits of the sheath, where its activity in the surrounding medium results in a steady progressive movement of the cell. Under the influence of somewhat violent stimuli, however, the sheath itself may undergo fibrations in such forms.

Owing to the nature of flagella and to their delicacy of structure, there are not many possibilities of variation in type. In addition to those which are circular or ellipsoidal in cross-section, there are some which are band form (some species of Dinoflagellida). Such band-form flagella suggest the possibility that vibratile membranes, which are not uncommon in parasitic types of flagellates, may, morphologically, be regarded as flagellum sheaths which remain attached

throughout their length to the cortex while the axial thread forms the contractile margin (Fig. 97, p. 212). Such vibratile membranes are characteristic of the genera *Trypanosoma, Cryptobia, Trichomonas, Trichomastix*, etc., all of which are parasites in the blood or digestive tract of different animals.

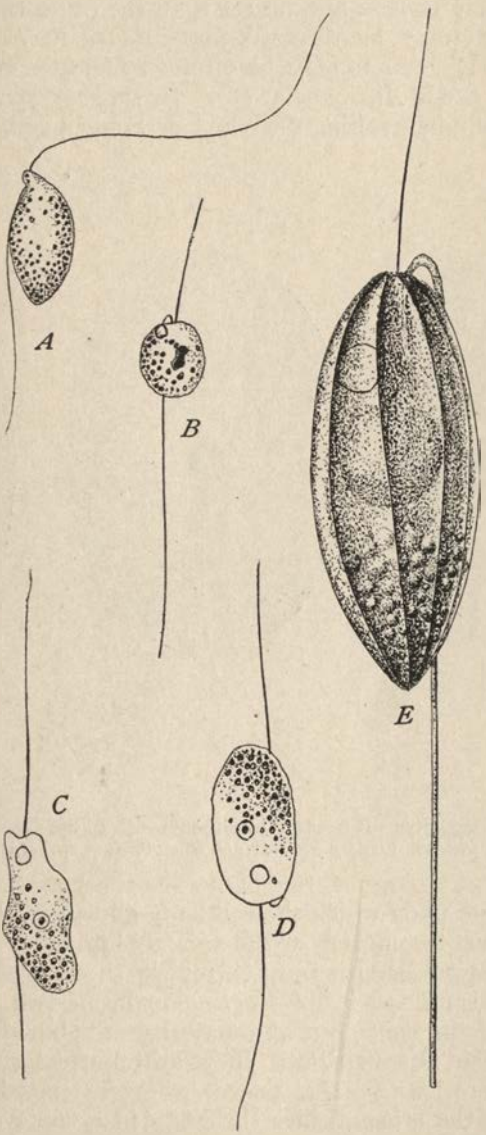

FIG. 69.—Free-living flagellates with trailing flagella. *A, C, D, Bodo caudatus* St.; *B, Bodo globosus* St.; *E, Plœotia vitrea* Duj. (After Calkins.)

There are, however, abundant variations in size, number, and position of flagella in the cell. When there is but one it usually emerges from a pit or funnel-shaped opening at the anterior end of the cell (flagellum fissure). When two are present they may be equal in size and length (*e. g.*, *Chilomonas paramecium*), or one may be considerably thicker and longer than the other (heteromastigote types). Both may be directed forwards as in Amphimonadidæ (Fig. 59, p. 117), or one may be directed forward, the other backward, as in *Bodo*, *Anisonema*, etc. In such cases the posteriorly directed flagellum (trailing flagellum or Schleppgeissel) appears to

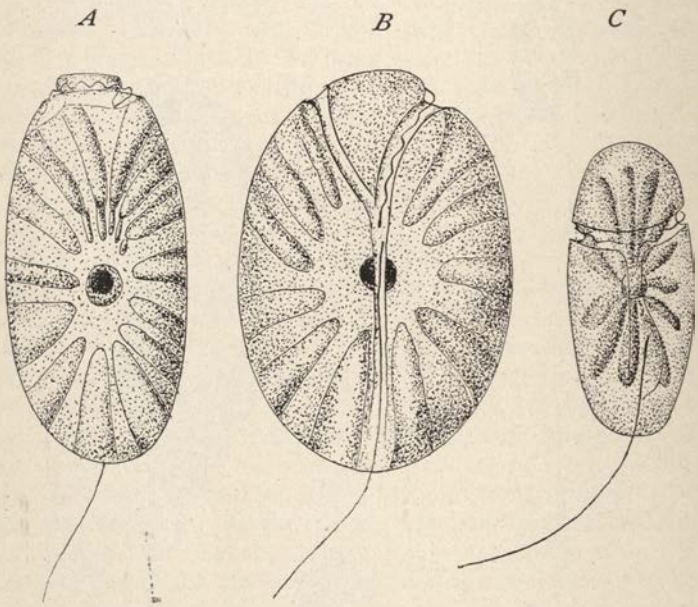

Fig. 70.—Dinoflagellates with reduced epithecæ. *A*, *B*, Side and ventral views of *Amphidinium herdmanni* Kof.; *C*, *Glenodinium* sp. (After Calkins.)

act as a runner upon which the cell body glides, and has little to do with the actual locomotion of the animal (Fig. 69).

Flagella vary in length from extremely short fibers in *Noctiluca miliaris* and some species of *Euglena* to forms two or three times the length of the body, but the majority are about as long as the body. In the Dinoflagellata they are particularly remarkable. Here there are two flagella, one of which extends freely into the water, while the other girdles the body in a transverse groove or annulus which is characteristic of these flagellates, where it gives the impression of many cilia (Fig. 70). The earlier figures of the Dino-

flagellates represent the furrow—which separates an epitheca or upper, from the hypotheca or lower part of the shell—with a fringe of cilia, and the name Cilioflagellata given to the group, indicates the extent to which this view prevailed. In some aberrant types like *Exuviælla marina*, in which there is no transverse groove, the "transverse" flagellum makes a sharp bend near the anterior end of the body and vibrates in a circle exactly as though it were still confined within a transverse groove (Fig. 71).

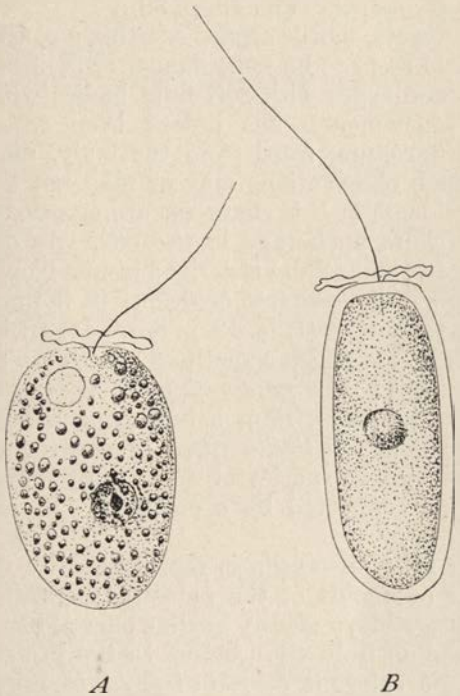

FIG. 71.—*Exuviælla marina* (*A*) and *E. lima* (*B*). (After Calkins.)

Delage and Hérouard have attempted to explain the dynamics of flagellum action whereby the comparatively heavy body is moved forward by reason of the vibrations of the exceedingly delicate thread. In the usual type the extremity of the flagellum describes a rather wide circle so that it is in a certain focus of the microscope for only an instant of time. With this circular movement, which varies in different species, constant undulations pass from the base to the tip. A forward pull results from the combination of such movements and the cell either glides smoothly after its active propeller, as in *Peranema trichophora*, or *Euglena oxyuris*, etc., or rotates more or less rapidly on its long axis while freely swimming

as in *Trachelomonas hispida, Euglena gracilis,* etc. When two flagella are present a curious shaking movement may accompany rotation and translation as in *Peridinium divergens* or other Dinoflagellida.

Only in rare cases are the flagella directed behind in swimming, the cell in such cases, like a spermatozoön, being pushed ahead by its motile organ. This divergent type of movement occurs when the usually sessile choanoflagellates are dislodged from their attachments and are forced to swim about. It is also characteristic of the marine flagellate *Oxyrrhis marina* (Fig. 43, p. 88).

With such energetic motile organs exerting a constant pull on the body there would seem to be some danger of their being pulled out, especially in those types with soft fluid bodies without firm periplasts. This phenomenon has indeed been recorded by some observers, the flagellum, freed from the body, moving off like a spirochæte. Such observations may or may not be well founded, at any rate accidents of this character are guarded against by the manner of flagellum anchorage in the cell. As described in the preceding Chapter, a flagellum is derived from a blepharoplast which may be just below the periplast or deeper in the protoplasm, or it may arise from the nucleus (Fig. 42, p. 86). Its anchorage is further assured by rhizoplasts which sometimes run to the posterior end of the cell as in *Chilomonas paramecium* or species of *Rhizomastix* (Fig. 46, p. 91), or which form a branching complex deep in the body substance as in *Mastigella vitrea* or *Astasia* species (Fig. 27, p. 65). In the various species of *Giardia* the basal bodies of the eight flagella are connected by a complete system of rhizoplasts (Fig. 140, p. 293).

Still another type of flagella is represented by the axostyles or internal motile organoids of the parasitic flagellates. In *Trichomonas* this appears like a glassy, hyaline curved bar of considerable diameter, extending from the nucleus to the posterior end of the cell where, like a spine, it projects from the periphery (Fig. 72). It is usually interpreted as a supporting axial rod to give rigidity of form to an otherwise soft and variable body (Doflein). Dobell regards it as a remnant of the centrodesmose left in the cell after division of the blepharoplast, a view supported by Hartmann and Chagas (1910) who interpret it as a centrodesmose formed during division of the intranuclear centriole. Martin and Robertson (1909), on the other hand, found that axostyles arise after division quite independently of the nucleus or of centrodesmose, and regarded them as independent organoids of the cell. Kofoid and his associates discard the assumption that axostyles are supporting or skeletal structures and place them in the category of kinetic elements. They are interpreted as intracellular organoids with a contractile function characteristic of flagella and serve as organs of

locomotion in the dense media in which the parasites live and in which the flagella would be ineffective. They are closely connected with the blepharoplasts in all species of *Giardia* (Fig. 140, p. 293), and are regarded as independent, self-perpetuating organoids which may be the first to divide in the processes of reproduction (*Giardia*) or the last to divide (*Trichomonas*). In all cases the axostyle divides longitudinally throughout its entire length, beginning with divisions of the anterior end in which the blepharoplast may be embedded (Fig. 72).

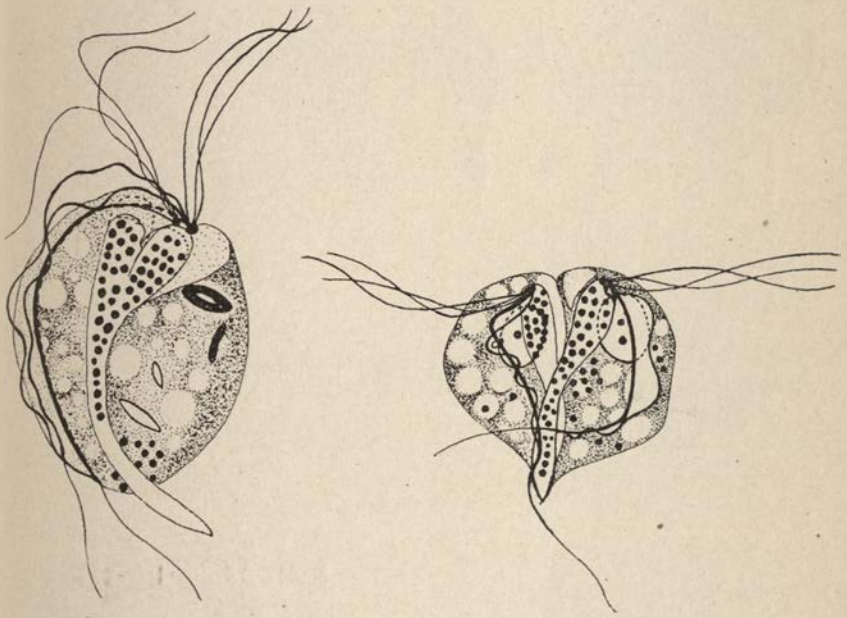

FIG. 72.—*Trichomonas augusta* Alex. Two successive stages in division of the axostyle. (After Kofoid and Swezy.)

In regard to the two opposing points of view as to the function of axostyles the weight of probability rests with the interpretation of Kofoid and Swezy (1915). The necessity of a supporting structure, or a form-rectifying organ, in these parasitic types is difficult to conceive. On the other hand their intimate relation to the blepharoplasts and their activity in reproduction indicate a common function with the kinetic elements. The observations of Kofoid and Swezy on the energetic movements of the axostyle while the organism works its way through the mucous afford a more plausible interpretation of the function of this organoid than the *a priori* views of those who see in such movements only the efforts of an elastic supporting structure to restore the form of a plastic cell.

2. **Pseudopodia.**— Pseudopodia are more or less temporary projections of the cortex which may serve for purposes of locomotion or, more often, as food-trapping or food-catching organoids. Four types are recognized, axopodia, rhizopodia (myxopodia), filopodia, and lobopodia, which differ widely in their structural make-up.

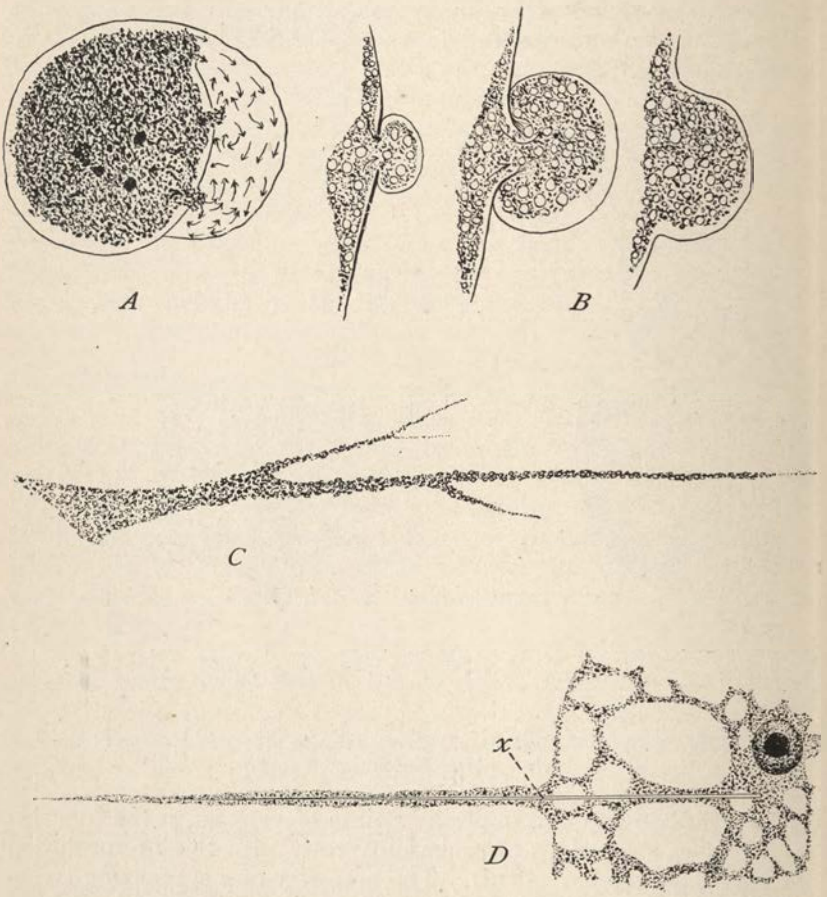

FIG. 73.—Types of pseudopodia. *A*, *B*, Eruptive type of lobopodium; *C*, myxopodia type of Foraminifera; *D*, axopodia type of Heliozoa. (After Calkins.)

Of these only the first type can be regarded in a strict sense as motile organs (see p. 133), the others functioning as food-catching organoids, or mere protrusions of the semifluid body.

Axopodia.— Axopodia are different from other types of pseudopodia in possessing, like flagella, central axial fibers of specialized protoplasm derived from endoplasmic kinetic elements. They are

found only in organisms belonging to the groups Heliozoa and Radiolaria, in which they radiate out in all directions from a usually spherical body (Fig. 73).

Unlike flagella, the outer coating of an axopodium is not a smooth periplast-like sheath, but consists of fluid protoplasm in which the movements of granules out on one side and back on the other are clearly discernible. In this manner the outer protoplasm is continually changing about the central axial filament, which alone is constant or fixed. Upon prolonged irritation, or in preparation for division or encystment, the axial filaments themselves, together with the enveloping protoplasm, are withdrawn.

Like flagella the axial filaments are formed as outgrowths from endoplasmic kinetic elements. *Gymnosphæra, Raphidiophrys, Sphærastrum, Acanthocystis, Dimorpha,* etc., possess characteristic "central granules" which, from their activities in cell division, are unmistakably centroblepharoplasts (see p. 99) from the substance of which the axial filaments are formed (Fig. 60, p. 117). *Wagnerella borealis,* in addition to the central granule, possesses a zone of basal bodies which give rise to the axial filaments and which at times of retraction of the pseudopodia are drawn into the central granule. In still other cases, as in *Actinosphærium eichhornii* the axial filaments do not arise, apparently, either from central granules or from nuclei, but appear to start indefinitely in the cytoplasmic reticulum (Fig. 73, *D*). In this case, however, the nuclei are so numerous that it would be difficult for an axial filament to escape proximity to some of them, while in a similar multinucleated form—*Camptonema nutans*—each nucleus gives rise to a single axial filament (Fig. 53, p. 102).

While the more common forms of Heliozoa are quiescent, floating types, some of the Heliozoa are freely motile. *Artodiscus* species, according to Penard, moves actively about, in the manner of a monad; *Acanthocystis aculeata,* as well as other species of the same genus, turns slowly over and over in a rolling movement; *Camptonema nutans,* according to Schaudinn, bends and straightens its axopodia in food-getting and in other activities. *Actinosphærium eichhornii* and *Actinophrys sol* are practically motionless. The active movements are due to the axopodia and the structure of axopodia is strikingly like that of flagella. That the contractile axial filament is the seat of this movement, and not the enveloping protoplasm is not open to reasonable doubt. Structure, function and mode of origin thus justify the inclusion of axopodia with the kinetic elements of the cell.

On the other hand, in types with axopodia which are practically motionless, the axial filaments have apparently lost the vibratile function and now serve as supporting elements for the long radiating pseudopodia. There is little reason to doubt that such elements are

homologous with the axopodia of motile types and that the latter are homologous with flagella. This is well illustrated by the case of *Dimorpha mutans* where two flagella and many axial filaments of axopodia originate from the same blepharoplast (Fig. 12, p. 34).

Speculations as to phylogeny on purely morphological grounds are not profitable, but in this group of Heliozoa we have pretty good evidence of a close relationship between flagellates and Sarcodina, and equally good evidence of the transition from an active kinetic element to an inactive, supporting axial rod, as seen in the pseudopodia of *Actinosphærium eichhornii*. This change in type is probably associated with the loss of specific kinetic centers for neither in the cytoplasm nor in the nuclei are such elements to be found. In some forms, finally, notably in *Clathrulina elegans*, the ends of the axopodia are frequently branched, a condition which points the way to pseudopodia of the rhizopodia type in which the supporting element is not in the form of an axial rod, but in the form of stiff stereoplasm (Fig. 73, *C*).

Rhizopodia.— This type of pseudopodia differs from others, first, in the tendency to branch, and second in the tendency to fuse or anastomose when such branches meet. From these characteristics they are sometimes called reticulose pseudopodia and myxopodia. So far as number of species is concerned, this type is the most characteristic form of Sarcodina pseudopodia. They occur in all forms of Foraminifera, Radiolaria and Mycetozoa which include the great majority of Protozoa. As a result of their unlimited power to branch and to anastomose, great meshworks of reticulated protoplasm are created which make ideal traps for the capture of food. In many types, especially in Radiolaria, they may be long and ray-like, with relatively little tendency to fuse; in other cases a main trunk gives rise to so many branches that it is lost in the reticulum, great accumulations of protoplasm collecting at the branching points (Fig. 9, p. 31).

Doflein includes axopodia and these branching anastomosing pseudopodia in the one type rhizopodia, and sees in the axial filament of the former and the inner protoplasm of the latter, only different states of the same fundamental stereoplasm. Axial filaments, however, derived from the substance of kinetic centers, are quite different from structureless axial stereoplasm which has no relation to kinetic elements. The enveloping protoplasm is apparently the same in both types and granule streaming is a common property, but the physical consistency is quite different. In rhizopodia the outer protoplasm is soft and miscible, leading to fusion on contact with one another, while axopodia never anastomose. The denser core of rhizopodia, while not condensed to a single fiber, serves the same function of support as the axial filament of *Actinosphærium* and gives stiffness and rigidity to long ray-like pseudo-

podia of many Foraminifera and Radiolaria which stand out in all directions from the cell.

Filopodia.—Structurally filopodia are entirely different from the types described above, being formed of clear hyaline ectoplasm in typical cases, or they contain a few granules indicative of endoplasm (Fig. 10, p. 32). They are usually long and slender and with rounded ends giving the impression of slender glass rods. In some forms there is a tendency to branch at the ends as in *Euglypha alveolata* (Fig. 8, p. 30), but there is never anastomosis. Sometimes they sway back and forth like a filament of Oscillaria, but usually they creep along the substratum where they serve mainly for food capture.

Filopodia are characteristic of the fresh water testate rhizopods, but are sometimes present in naked types like *Amœba radiosa* or *Amœba actinopoda* (*?*).

Lobopodia.— Lobopodia are made up of granular endoplasm and hyaline ectoplasm, and are temporarily projected portions of the body protoplasm not to be compared with definite locomotor organs of other Protozoa. The inner protoplasm of nearly all kinds of Protozoa with granules of various kinds, food substances more or less digested, and waste materials, is in constant movement called cyclosis. In more highly differentiated forms, and in organisms with a firm cell membrane, this movement is confined to the internal protoplasm and the form of the cell is not affected by it. In the shell-less rhizopods, however, there is no such outer covering, and the peripheral protoplasm gives way at the weakest points, and an outward flow of protoplasm with corresponding change in the form of the body results (see Chapter IV). If such a weak point is constant in position, a constant flow in its direction is the outcome, and the *Amœba*, consisting of practically one pseudopodium, as in the *limax* types, moves in one direction (Fig. 29, p. 67). In *Amœba verrucosa* a delicate periplast surrounds a somewhat dense protoplasm which, accumulating on one side (according to Rhumbler 1898), causes the cell to roll over.

Withdrawal of pseudopodia is accomplished by their absorption into the body substance, and is accompanied by a wrinkling of the denser ectoplasm preparatory to its transformation into endoplasm (see Schaeffer).

In pseudopodia generally it is evident that we have to do with different types of structure which, in only a few instances, can be regarded as motile organs. Axopodia, with their axial filaments derived from kinetic elements, are closely related to flagella and may be regarded as organs of locomotion, but the other types, which may represent highly modified axopodia, have lost the kinetic elements, if they ever had them, and are useful only as food-catching organs. In most rhizopods the entire organism is the motile element, rhizo-

podia, filopodia and lobopodia being expressions of energy transformations comparable with the rotation of protoplasm in Nitella or circulation in Tradescantia. Axopodia of the motile Heliozoa, axial filaments of the inactive species, and stereoplasmic cores of the rhizopodia, may be regarded as successive phases in the modification of vibratile flagella. These types of pseudopodia have in common an enveloping layer of granular protoplasm, but filopodia and lobopodia represent a different type, being made up in large part, or entirely, of ectoplasm and without any evidence whatsoever of kinetic elements. So-called "contractile elements" of this type of pseudopodia are largely figments of the imagination.

3. **Cilia.**—Cilia are the motile organs of Infusoria and accompany the most highly differentiated types of cortex to be found in the Protozoa. Individually they are shorter, more delicate and less powerful than flagella and owe their importance as motile organs to their large numbers and synchronous beating. Their action may be compared with that of oars in rowing, while flagellum action might be compared with sculling, and the results of cilia and flagella activities bear a relation similar to that between a racing shell and a gondola.

According to the interpretation of several observers, mainly Schuberg, Maier, Schubotz, Schröder, etc., the cortex of ciliates is a composite of zones of differentiated protoplasm. In the majority of cases such zones cannot be made out, for one shades into the other, and the whole into the alveolar endoplasm. In favorable cases, however, we can distinguish (1) a superficial periplast perforated for the exit of cilia and trichocysts when present; (2) an alveolar layer containing trichocysts if the latter are present; (3) a contractile zone containing the basal bodies of cilia, myonemes and coördinating fibers; (4) a denser zone which shades off into the endoplasm and supplies an anchorage for nuclei and contractile vacuoles.

A single cilium is constructed on much the same plan as a flagellum, consisting of a central axial filament or fiber, and an elastic sheath of protoplasm. Movement is due to the active contraction in one plane of the axial fiber and recovery to the elasticity of the enveloping sheath. The contractile element originates from a basal body in the contractile zone. In *Pycnothrix monocystoides*, according to Schubotz (1908), there are two basal bodies, one internal, which are connected by a rhizoplast. Connecting these basal bodies is a series of longitudinal and transverse coördinating fibrils (Fig. 54, p. 105).

The arrangement of cilia on the surface of the body varies in different species; sometimes they form a complete coating for the organism as in the majority of Holotrichida (Fig. 76); sometimes they are limited to certain zones as in *Urocentrum turbo*, *Didinium nasutum*, etc. (Fig. 168, p. 383); or sometimes to the ventral surface,

as in generalized Hypotrichida (Fig. 79). In all cases they are arranged in longer or shorter rows running straight or spirally, and giving the striped appearance characteristic of the ciliates. Waves of contraction pass from the anterior end posteriorly, cilia of the same transverse rows beating synchronously, those of the same longitudinal rows metachronously.

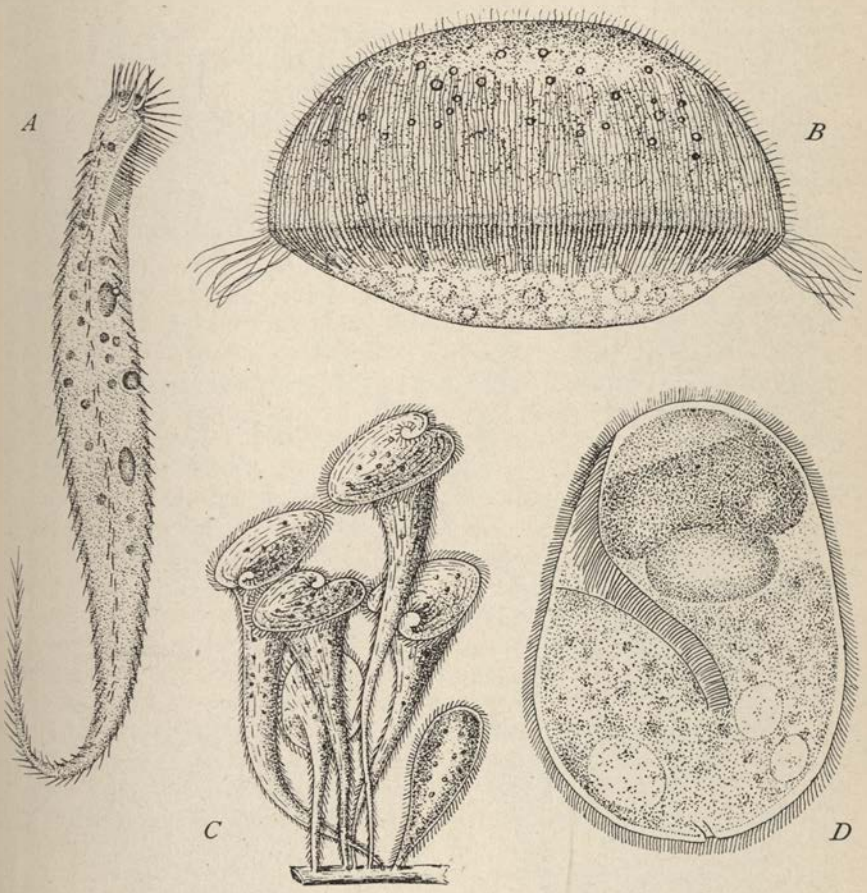

FIG. 74.—Types of Ciliata. *A*, *Uroleptus pisces* (after Stein); *B*, *Cyclotrichium gigas* (after Fauré-Fremiet); *C*, *Stentor polymorpha* (after Bütschli); *D*, *Nyctotherus ovalis*. (Original.)

The periplast is variously sculptured in different species, giving the appearance superficially of a different mode of origin of the cilia. In some cases they appear to come from the centers of minute cups or dimples as in *Paramecium aurelia;* in other cases from longitudinal grooves or furrows between ridges of periplast

(Fig. 54, p. 105), and in some they appear to come from the ridges themselves.

Rhizoplasts or endoplasmic prolongations from the basal bodies, are comparatively rare but occur in some cases as in *Didinium*

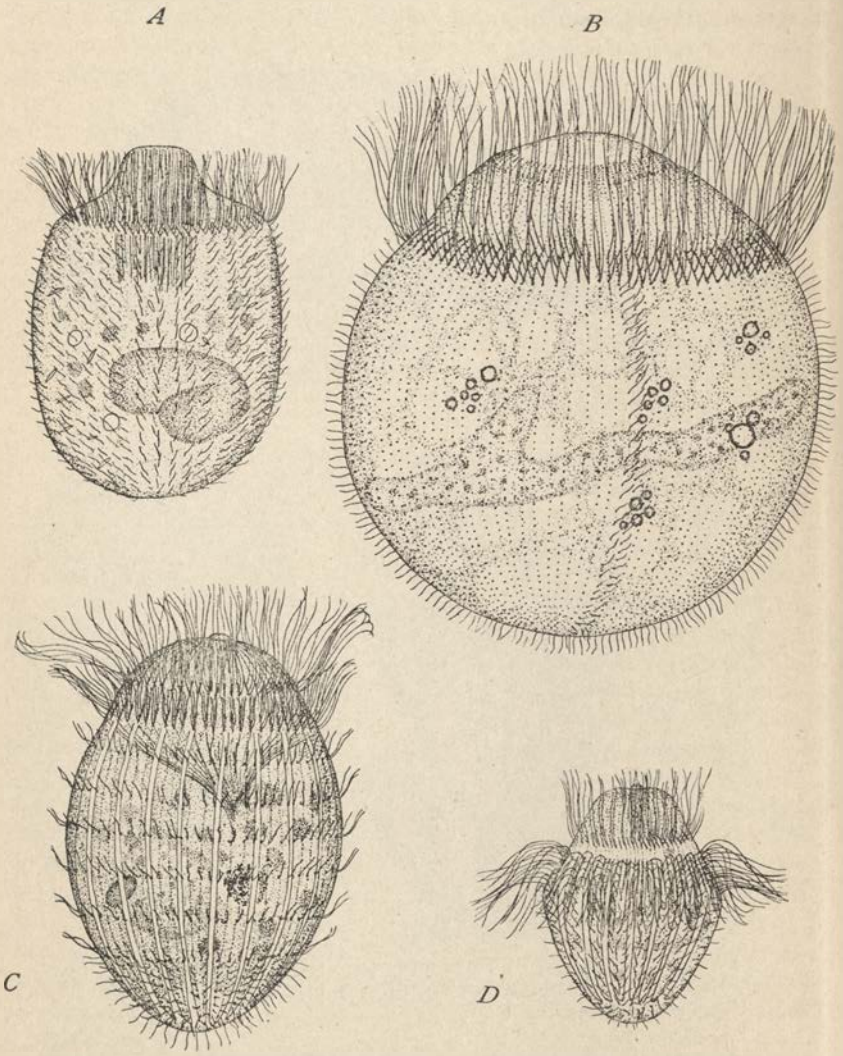

Fig. 75.—Types of Ciliata. *A, Monodinium balbianii; B, Cyclotrichium sphæricum; C, Dinophrya lieberkühni; D, Askenasia elegans.* (After Fauré-Fremiet.)

nasutum (Fig. 89, p. 180). Coördinating fibrils have been described in a few types (*Euplotes*, *Diplodinium*, *Balantidium coli* and *B.*

suis, see p. 108), and center in a specialized neuromotor body, the motorium (Yocom, Taylor, Sharp).

In some cases cilia are uniform in length over the entire body (*Opalina*); in other cases they are longer in the region of the mouth or around the posterior end, but no sharp dividing point separates short from long ones (Fig. 75). In some cases they are uniformly long and vibrate like flagella (*Actinobolus radians*, Fig. 81, p. 154).

4. **Composite Motile Organs.**—A well-marked characteristic of cilia is the ability of two or more to fuse into motile organs of variable complexity. Such combinations give rise to membranulæ, membranelles, undulating membranes and cirri, each of which, although composed of fused cilia, originates or grows as an independent and complete organoid. In each case also the component cilia may be demonstrated by use of dilute alkalies such as potassium or sodium hydrate.

Membranulæ.—Membranulæ are very long, delicate, finely-pointed aggregates of cilia which differ from the somewhat similar cirri in movement and in composition, while their basal granules, in *Didinium nasutum* at least, are connected with the vicinity of the nucleus by definite rhizoplasts (Fig. 89, p. 180). Similar membranulæ form the basal ring in Vorticellidæ (Schröder, Schuberg, etc.).

Membranelles.—Membranelles are formed by the fusion of cilia in the region of the mouth. In many of the Holotrichida the cilia are longer just posterior to the mouth than in other regions of the body, frequently forming circlets about the mouth as in *Lacrymaria olor* or *L. lagenula* (Fig. 76). In the other Orders of Ciliata oral cilia are fused to form membranelles. In the oral regions the body is usually differentiated into a specialized food-collecting, frequently funnel-like structure called the peristome. Cilia on the floor of the peristome are usually longer than in other parts of the body, and in four of the five orders of ciliates some of these are invariably aggregated in triangular, quadrilateral or ribbon-like membranelles and membranes for producing food-bringing currents of water toward the mouth. In every order except the Holotrichida a fringe of such specialized motile organs, known as the *adoral zone*, lies on the left margin of the peristome (Fig. 78).

Membranelles are usually made up by the fusion of two rows of cilia as shown by the double row of basal bodies (Maier) and their flat or curved faces make powerful sweeps in the water (Fig. 78, p. 150). According to Schuberg, Gruber, Maier and others, the anchorage of these organoids is quite complex. The basal granules form a double row immediately below the periplast; fibrils from these, analogous to rhizoplasts, form a broad triangular basal plate and are then brought together to form an end thread which connects the membranelle with coördinating fibers (Fig. 57, p. 110).

In some types the anterior membranelles fold over the peristome forming an operculum as in *Uronychia setigera* (Fig. 107, p. 225).

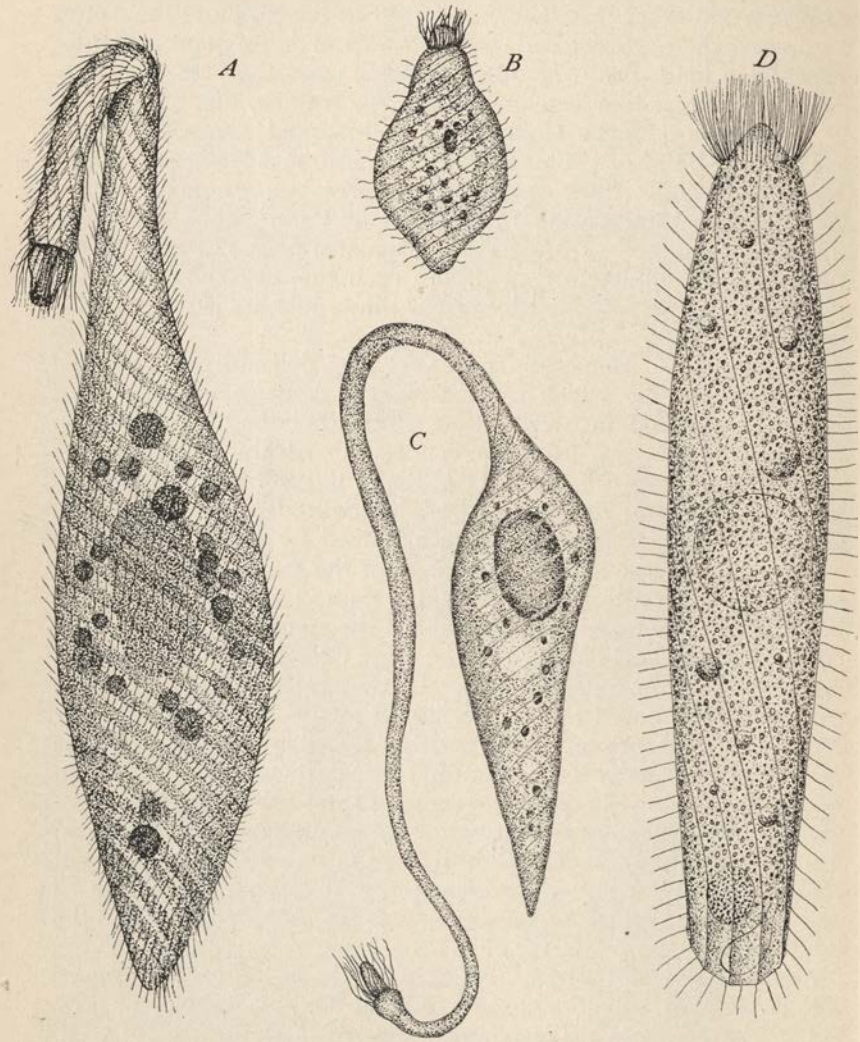

FIG. 76.—Types of *Lacrymaria*. *A*, *Lacrymaria* sp.: *B*, and *C*, retracted and expanded phases of *Lacrymaria olor; D*, *Lacrymaria lagenula*. (After Calkins.)

While in most cases the membranelles represent the fusion of comparatively few cilia in transverse rows of the peristome, making them relatively narrow at the base, in other cases, notably in the Tintinnidæ, such fusion includes practically all of the cilia of the

transverse rows, making membranelles as broad as the peristome (Fig. 174, p. 391). In the Vorticellidæ there are two rows of membranelles the double adoral zone winding about the peristome usually in a direction opposite to that of the Heterotrichida and Hypotrichida (Fig. 78, p. 150).

Undulating Membranes.—Undulating membranes are found in all orders of the ciliates and range in size from delicate aggregates no broader from base to tip than ordinary cilia to relatively enormous balloon-like structures equal in width to more than half the diameter

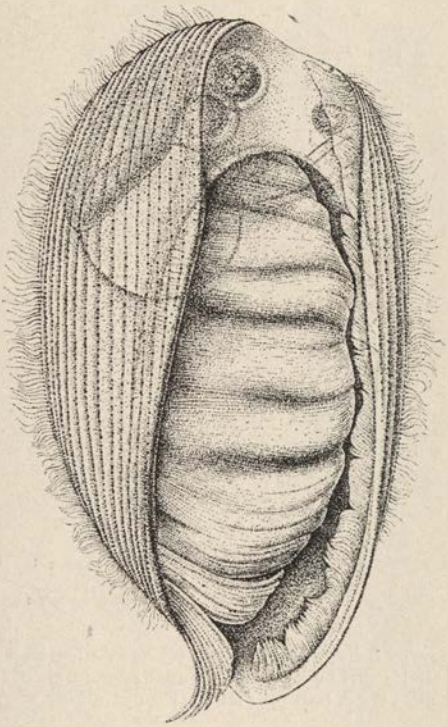

FIG. 77.—*Lembadion conchoides* F. F. (After Fauré-Fremiet.)

of the body, and in some cases as *Lembadion conchoides*, almost equal to length of the body (Fig. 77). In the simplest cases these membranes are composed of a single row of longitudinally placed cilia, the basal bodies of which form a single basal strand. Since cilia of the longitudinal rows beat metachronously the result of their contraction when fused in these undulating membranes is a series of waves passing from the anterior to the posterior end. In more complex forms undulating membranes may be composed of 3 to 10 rows of cilia, fused in longitudinal rows, the length varying from a few

microns to great waving sheets of protoplasm almost as long as the entire cell (Fig. 77). They are usually found in the peristomial area inside the rows of membranelles or adoral zone and are named preoral, endoral, paroral, etc., according to their positions in relation to the mouth (Fig. 78). They are also frequently found in the gullet, a single one, for example, in *Paramecium*, two in *Glaucoma*, etc., and are used exclusively for food-getting (see Maier, 1903), Schuberg, 1905, etc.).

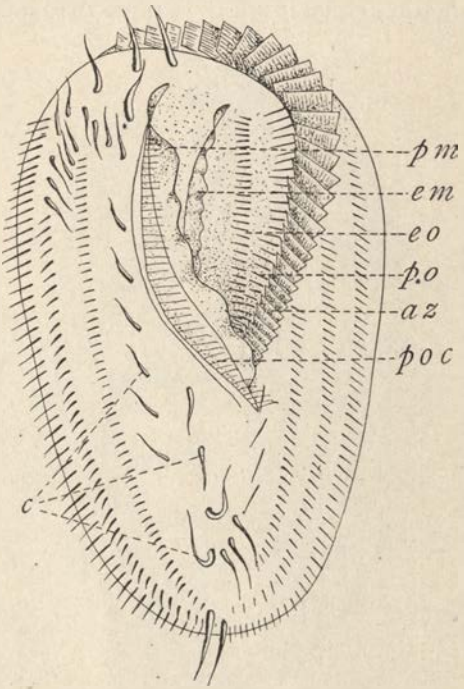

FIG. 78.—Diagram of a hypotrichous ciliate. *a z*, adoral zone of membranelles; *c*, anal and ventral cirri; *e m*, endoral membrane; *e o*, endoral cilia; *p m*, paroral membrane; *p o*, preoral cilia; *p o c*, paroral cilia. (From Calkins.)

Cirri.— Cirri are the most highly specialized of all the motile organs of ciliates, the most characteristic forms occurring in the Hypotrichida. They are placed more or less definitely on the ventral surface, a group, variable in number, at the anterior end being known as the frontal cirri, a similar group, also variable in number, near the posterior end being known as the anal cirri, while other groups may form caudal cirri, ventral cirri, marginal cirri, etc. (Figs. 78, 79).

Cirri are always broader at the base and taper gracefully to a fine point. In cross-section near the base they are either circular,

ellipsoidal, quadrilateral or irregular, and always have a basal plate made up of the basal granules of the fused cilia. Under unfavorable conditions of the medium in which the organisms live, and usually after imperfect fixation, the constituent cilia become separated particularly near the tip, and the cirri then present a most frayed-

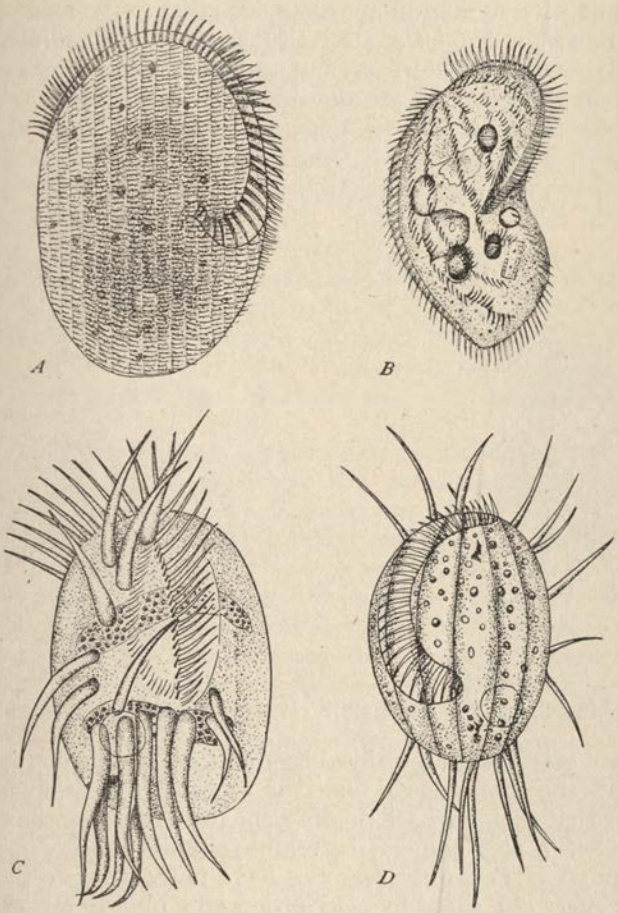

FIG. 79.—Types of hypotrichous ciliates. *A*, *Peritromus emmæ; B*, *Kerona pediculus; C*, *Diophrys appendiculatus; D*, *Euplotes charon*. (*A*, *C*, *D*, after Calkins; *B*, after Stein.)

out or ragged appearance. They vary in size from extremely minute cilia-like marginal cirri to great ventral brushes in forms like *Onychaspis* (Fig. 80) or huge hooked structures as in *Uronychia* and other Euplotidæ (Fig. 79).

Cirri are preëminently organs of locomotion, but, unlike other

motile organs of the ciliates, their stroke is not confined to one plane but may be in any direction. This gives to the Hypotrichida an extreme variety of movements unparalleled by any other group of Protozoa. Many of them walk or run on the tips of their frontal and ventral cirri (*Stylonychia*); others swim with a peculiar jerky movement (*Aspidisca*); others combine swimming due to the adoral zone with sudden jumps or springs due to the anal or caudal cirri (*Uronychia, Euplotes*, etc.). Such saltations are not limited to the Hypotrichida, however, but are characteristic of organisms in all groups where cirri are developed as in *Halteria grandinella* among Oligotrichida, *Mesodinium cinctum* among Holotrichida, etc.

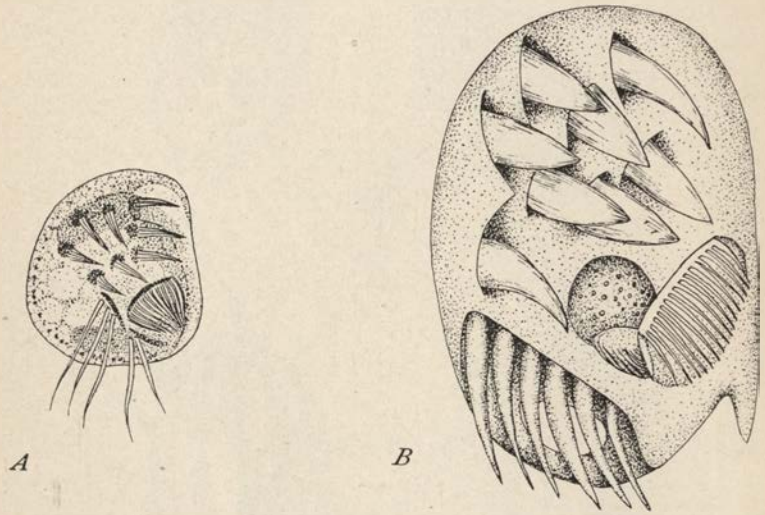

FIG. 80.—*A*, *Onychaspis* sp.; *B*, *Onychaspis hexeris*. (Original.)

In some cases cirri are said to be differentiated as tactile organs, especially the more dorsal ones of certain Hypotrichida. It is probable that such cirri are no different from other motile organs of the ciliates in this respect, extreme irritability being a common characteristic. Few observers can have failed to note the instantaneous effect of a slight local irritation on a quietly resting *Pleuronema chrysalis*, for example, with its long cilia radiating out in all directions, yet there are no cirri here.

The synchronous and metachronous vibrations of cilia and cilia aggregates, are probably regulated by coördinating fibers with highly developed irritability. This is the interpretation given by Schuberg to the basal fibrils in the contractile zone of *Paramecium caudatum;* by Nerescheimer (1903) to certain fibers distinct from the myonemes in *Stentor cœruleus*, and by Sharp, Yocom, Taylor

and others, to conspicuous fibers in *Diplodinium ecaudatum* and *Euplotes patella* (see p. 112). In the latter organism Yocom (1918) and Taylor (1920) found fibers running from the posterior anal cirri and from the adoral zone of membranelles to a common anteriorly placed structure termed the motorium, which they regard, with Sharp (1914) as a center of the neuromotor system (see p. 109). The ventral and frontal cirri, however, are not connected by similar fibrils with this motorium, but possess bundles of fibrils, described earlier by Prowazek in *Euplotes harpa*, and by Griffin in *E. worcesteri*, which may run in any direction until lost in the endoplasm (Fig. 79, p. 151). The inference is that these cirri are independent of the coördinated system of fibrils which regulate the adoral zone and the anal cirri, and that their movements, which are always irregular, are not affected by cutting the coördinating fibrils of the motor system (Fig. 57, p. 110, also see p. 112).

(*c*) **Other Organoids Adapted for Food-getting.**—Mention may be made here of a few special types of cortical differentiation apart from the cell mouths, which Infusoria use for purposes of food-getting. The most striking of these are the tentacles of *Actinobolus radians*, the "tongue" or "seizing organ" of *Didinium nasutum* and the tentacles of the Suctoria.

Contractility due to myonemes is a widely-distributed phenomenon in ciliated Protozoa and in most cases involves the activity of the entire organism (see p. 105). When it is limited to restricted portions of the body, such as the peristomial complex of *Diplodinium ecaudatum*, or the "vestibule" of Vorticellidæ, it acquires a special interest. Even more remarkable than these, however, is the power, possessed by *Lacrymaria olor*, of projecting its mouth-bearing extremity any distance up to three times the length of the flask-shaped body, or until the rubber-like neck is reduced to a mere fibril. The "head" thus projected dashes here and there with amazing rapidity, the body meantime remaining quiet and unmoved, until finally the head and neck are withdrawn and the cell swims off with no visible trace of contractile structures (Fig. 76, p. 148). No special myonemes have been described in this form and the projection and retraction of the "head" must be due to the elasticity of the cortex of the "neck" region, combined with activity of the oral circlet of cilia while the body cilia are at rest.

Another remarkable and special phenomenon, seen apparently by few observers, is the method of food-getting by *Actinobolus radians*. This organism, when at rest, protrudes a forest of radiating tentacles which stand out like axopodia, sometimes stretching a distance equal to several times the body diameter. The ends of these tentacles carry trichocysts (Entz, Calkins, Moody) which upon penetrating an individual *Halteria grandinella*, completely paralyze it.

The tentacle, then, with prey attached, is withdrawn entirely into the body, the *Halteria* is worked around to the mouth and swallowed (Fig. 81).

In *Didinium nasutum* the proboscis bears a peculiar protrusible plug or tongue of protoplasm termed the "seizing organ" by Thon (1905) and Prandtl (1907) (Fig. 89, 8). A zone of trichocyst-like fibrils lies near the extremity of this plug and when certain types of ciliates, preferably *Paramecium*, are struck by *Didinium* the plug, with trichocysts, is shot out penetrating the cortex of the prey and paralyzing it. While this process takes place too rapidly to be seen the results show that it must have taken place for, after striking and anchoring in the *Paramecium*, the seizing organ with prey

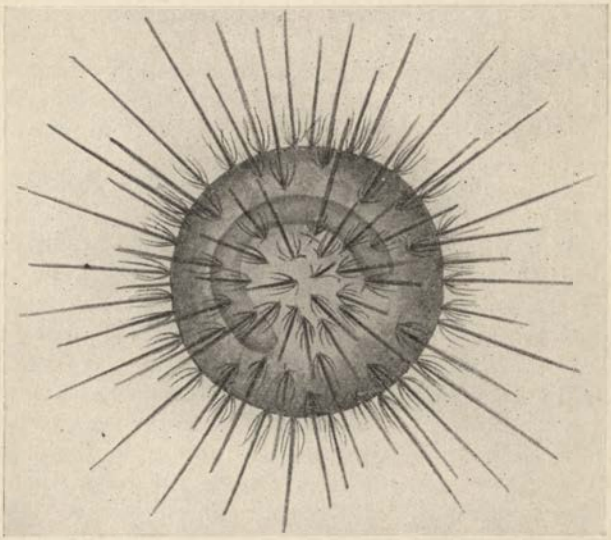

FIG. 81.—*Actinobolus radians* St. (After Moody.)

attached is retracted and the prey, often larger than the captor, is swallowed whole (Fig. 89). No satisfactory explanation of this phenomenon has yet been given.

Still another type of cortical organs is illustrated by the various kinds of tentacles of the Suctoria. Some of these are constructed for piercing, while others are hollow, forming sucking tubes through which food is taken into the body. They are evidently provided with some type of poison for active ciliates, coming in contact with these tentacles, become suddenly quiet and remain so while the suctorial tentacles penetrate the cortex and suck out the endoplasm of the prey which can be followed through the feeding tubes to the endoplasm of the captor (Maupas, 1883). Like the tentacles of

Actinobolus radians, these suctorial tentacles are retractile, but again there is no satisfactory explanation of their activity and no description or mention of specialized motile apparatus.

Like the majority of formed organoids of the cell the more complicated of the motile organs described above are formed anew at each division of the cell. This does not apply to the majority of pseudopodia nor has it been observed in the case of cilia, but is well-established for flagella and for the aggregates of cilia, such as membranelles, undulating membranes and cirri. In a few cases the flagella themselves are said to divide, but this is questionable, the flagella probably arising in all cases from the substance of blepharoplasts or basal bodies which have divided. Young (1922) has recently shown that a cirrus of *Uronychia transfuga* if cut does not regenerate, but if the protoplasm is partly included in the operation a new cirrus is regenerated. Demboska has still more recently (1925) shown that if a single cirrus of *Stylonychia* is cut out all of the cirri are renewed.

(*d*) **Oral and Anal Cortical Modifications.**—In all naked forms of Protozoa and in corticate forms which manufacture their own food as in the phytoflagellates or which, like *Opalina*, take in food substances by osmosis through the general body surface, there are no portions of the ectoplasm differentiated as cytostomes or cell mouths. In such forms, furthermore, where there is no undigestible matter, there is no modification as cytopyge, or cytoproct, or cell anus. In testate forms, obviously, there is only a limited region of the body substance which is open for the reception of food. In testate rhizopods the shell openings are due to the physical conditions under which the lifeless shell materials are deposited and no definite mouth parts as protoplasmic differentiations are present.

In all Protozoa, on the other hand, which take solid food and which are covered by more or less highly differentiated cortical plasm, there are permanent openings in the cortex serving for the intake of solid bodies and for defecation of undigested remains. In many cases such openings in the cortex merely expose a limited region of soft receptive protoplasm as in *Oikomonas termo* (Fig. 88, *B*), but in other cases complicated cortical differentiations with supporting and food-procuring adaptations give rise to complex and permanent cytostomes and cytoprocts.

In flagellates such an area of softer protoplasm is situated at or near the base of the flagellum, or two such areas may be present, each at the base of a flagellum or group of flagella, as in *Trepomonas* and *Hexamitus* (Fig. 88, p. 179). In one group, the Choanoflagellidæ, a collar-like membrane, arises as a protoplasmic fold around the base of the flagellum and forms a cuff or funnel surrounding the flagellum for a distance equal to one-third or one-half its length (Fig. 82). These are extremely delicate, the margins alone in

many cases indicating their presence and dimensions. According to Françe they are somewhat spirally rolled like a cornucopia, the free margin arising from the softer food receptive area and by its movements directing food particles toward this area. In some cases two such collars, one within the other, are present as in

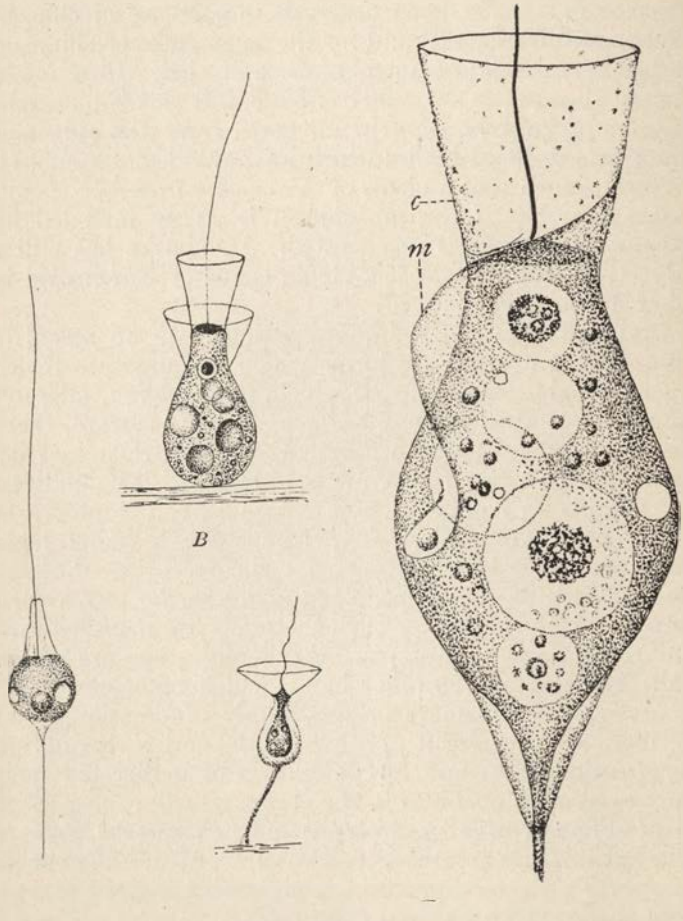

FIG. 82.—Types of choanoflagellates. *A*, *Codosiga pulcherrimus; B*, *Diplosiga socialis, C*, *Salpingœca marinus; D*, Collar type according to Françe. (After Calkins.)

Salpingœca entzii or *S. marinus* (Fig. 82). The second, outer, collar here is regarded by Doflein as a periplastic rigid structure which forms a part of the cup or house and is not morphologically equivalent to the inner collar, which, like a pseudopodium, may be shortened or lenthened, or drawn in and formed anew by the living

cell. According to the older interpretation these protoplasmic collars assist in food-taking by forming a sticky directive course for particles down the inside to the receptive area at the base of the flagellum (Kent), but according to Françê granules on the inside of the collar are moving away from the cell as defecatory material while the food particles move down the outside to a receptive area not included by the collar base (Fig. 82, *D*).

In the majority of corticate flagellates the food-taking receptive area is continued as a pit or groove known as the flagellum fissure, or as the cytopharynx. The flagellum arises usually at or near the base of such a pit and in many cases the contractile vacuole empties into it (Euglenida, etc., Figs, 85, 95).

It is in the ciliate group, however, that we find the most characteristic and most complicated types of cytostome. Here they may be mere pores in the cortex which remain closed except during the process of ingestion and without accessory current-producing motile organs, or they may be permanently open and provided with undulating membranes or other vibratile elements. The former type, known as the Gymnostomina, eat only occasionally and then by a definite swallowing process, the soft mouth region widening into a huge opening to receive the prey. Thus *Didinium nasutum* ordinarily swims about with little evidence of a mouth at the extremity of the conical proboscis (Fig. 88, *C*), but when swallowing a *Paramecium* which may be larger than itself, the entire anterior end appears to be nothing but mouth, the body wall of the *Didinium* being reduced to a thin enveloping sheath about the *Paramecium* (Fig. 89). Similar, but not so spectacular cytostomes are present in other types of Gymnostomina. *Spathidium spathula* may swallow smaller ciliates like *Colpidium* (Fig. 90), *Nassula aurea, Chilodon cucullulus*, etc., still smaller forms (Fig. 88, p. 179). The Trichostomina are always provided with food-getting motile organs and a constant stream of water with suspended bacteria and other minute living things passes through the permanently open mouths making these creatures, according to Maupas, gluttons *par excellence* of the animal kingdom.

The complications in regard to structure in these two types of cytostome have to do with the support of the walls of the mouth and of the gullet into which the mouth opens, and for the perfection of the current-producing apparatus. Such support is obviously important in preventing rupture of the soft protoplasmic bodies of forms like *Didinium nasutum, Enchelys farcimen Prorodon teres* or *Spathidium spathula* (Fig. 89, p. 180). In all of these cases there is an armature of elongated rods, called trichites, formed of stereoplasmic substances, embedded in the walls of the mouth and gullet, and these, like spiles in a ferry slip, take up the strain when the mouth is opened. In many cases, however, the perfection and

strength of these cytostomial supports seem to be entirely out of proportion to such hypothetical needs of the organism. Thus in all of the Chlamydodontidæ the trichites form a tubular armature, the ends making a circumoral ring which may project beyond the ventral surface (*Chilodon cucullus*). Such an aggregate, known as an oral or pharyngeal basket, or pharyngeal armature, forms a more or less definite cytopharynx. In some cases the trichites are replaced by a compact corneus tube which extends deep into the the endoplasm as in *Nassula aurea, Orthodon hamatus, Trachelius ovum*, etc. (Fig. 83).

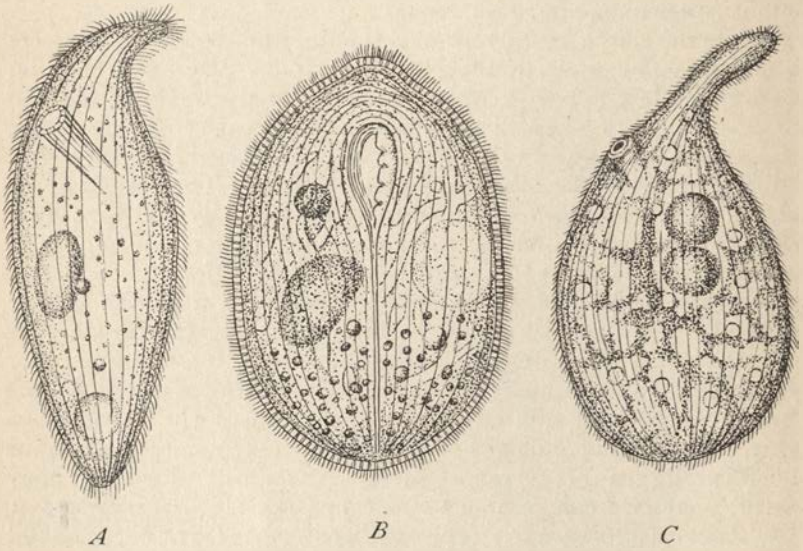

FIG. 83.—*A*, *Orthodon hamatus* with oral tube; *B*, *Frontonia leucas*. with undulating membrane on left margin of mouth; *C*, *Trachelius ovum*. (*A* and *C*, after Bütschli; *B*, after Calkins.)

In the Trichostomina the permanently open mouth always leads into a more or less highly-developed gullet or cytopharynx, while peristomial cortical differentiations of various kinds lead to it. The cytopharynx is usually provided with one or more undulating membranes, while membranelles, undulating membranes and cirri may also be present in the peristome (Fig. 78, p. 150).

The mouth region of the ciliates appears to be the focal point of the longitudinal rows of cilia. In the generalized forms, such as *Actinobolus radians, Prorodon teres, Holophrya discolor*, etc., the mouth is exactly terminal and the rows of cilia run symmetrically to the posterior end (Fig. 165, p. 378). In the majority of cases, however, the mouth is not terminal but may be found at various

points on the side or upon the ventral surface. Thus it may be on the side in forms like *Nassula aurea*, or *Dallasia frontina* (Fig. 168, p. 383), on the ventral anterior surface in *Frontonia leucas*, or various species of *Chilodon* (Fig. 34, p. 77), or at the extreme posterior end as in *Opisthodon mnemiensis* (Fig. 166, p. 379). Wherever the mouth is found the rows of cilia are correspondingly altered from symmetrically-placed lines as in the generalized forms, to all kinds of asymmetrical arrangements. This has led to the view, first elaborated by Bütschli that the ancestral position of the mouth in ciliates, was terminal at the anterior end, and that, in response to requirements of different modes of life, and to various types of food, the mouth has shifted from the anterior end to the various positions as now found in different types. With this shifting the focal points of the ciliary rows have similarly shifted, and the positions of the lines of cilia in some forms are used as evidence to indicate the path of this shifting and the mode of evolution of the present-day cytostomes. A familiar illustration of such shifting is the series of forms represented by the genera *Holophrya*, with terminal mouth, *Spathidium*, with oblique mouth, *Prorodon* and *Dallasia*, with subterminal mouths, *Amphileptus* and *Lionotus* with elongated slit-like mouths extending from the anterior end far down the ventral surface, such types leading to the various proboscis-bearing genera like *Dileptus* in which the mouth is limited to the posterior end of such an ancestral slit-like aperture, now represented for the most part by a row of trichocysts (Figs. 157, 166, 167).

In *Chilodon* there is an oblique line of cilia running from the anterior left-hand margin of the ventral surface to the circular mouth which in some species may be shifted well over on the right side. The lines of ventral cilia begin at this line and not at the mouth, while an oblique row of specialized cilia suggests the beginnings of adoral zone formations characteristic of the majority of Trichostomina.

In many types of ciliates, a special region of the body, not found in the more generalized forms, is developed as a feeding surface. Such regions, known as *frontal fields*, are characteristic of ciliates which live permanently or temporarily as attached forms. There is some evidence to indicate that such frontal fields as occur in *Stentor*, and the Peritrichida, are derived from the anterior ventral surface of more actively moving forms. In *Peritromus*, for example, the line of the peristome cuts out a definitely limited frontal region of the ventral surface, which is provided with special motile organs, the frontal cilia. Bütschli (1888) suggested that such a peristome, if continued around the right side of the organism would completely separate an anterior frontal field from the remainder of the body, as seems to be the case in *Climacostomum virens* (Fig. 56, p. 107). With the development of an attaching portion of the body as in

Stentor, and in the interest of feeding, such a frontal field becomes directed upward, reaching its most perfect development in types like *Vorticella* and its allies (Fig. 74, p. 145).

Such frontal fields are flat in the various species of *Stentor*, or they may be greatly invaginated as in *Bursaria truncatella*, or drawn out into ciliated food-getting arms as in *Folliculina ampulla*, or rolled up in spiral folds as in *Spirochona gemmipara* (Fig. 84).

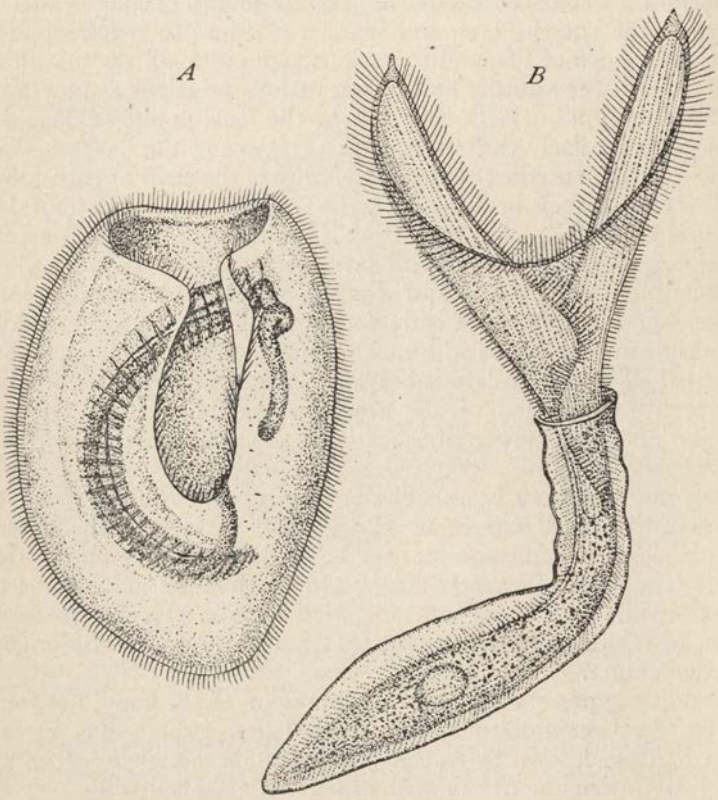

FIG. 84.—*A*, *Bursaria truncatella*, frontal field deeply insunk; *B*, *Folliculina ampulla*, with frontal field drawn out into two flexible arms. (*A*, original; *B*, after Doflein.)

The cytoproct is rarely differentiated as a definite opening in the cortex. In many cases, especially in the flagellate group, the cytopharynx and anus are the same. In the majority of ciliates, on the other hand, there is a constant opening or pore, usually in the posterior region of the body, which is closed and invisible except during the process of defecation (Fig. 30, p. 70). In some forms, notably

in *Pycnothrix monocystoides* and *Diplodinium ecaudatum*, a definite anal apparatus is developed. In the latter case Sharp describes a "rectum" with distinct walls opening to the outside by a permanent cytopyge, while at the inner end there is a "cecum" which acts as a collecting vacuole for the fecal matter (Fig. 2, p. 20).

(*e*) **Contractile Vacuoles.**—In the rhizopods and most of the soft-bodied flagellates the contractile vacuole can scarcely be called a cortical differentiation. In these cases they are more or less casual organoids, moving freely with the endoplasmic granules. In the corticate flagellates and ciliates, however, there is a permanent spot in the cortex through which the contents of contractile vacuoles, fixed in position, are emptied to the outside. As a rule the salt water forms of Protozoa do not have contractile vacuoles (see p. 168) and the number in fresh-water forms is variable, sometimes in the same organism (testate rhizopods and Heliozoa). In many types, however, the number as well as the position is fixed; two as a rule in the phytoflagellates, one in Hypotrichida and Peritrichida, and variable numbers in the Holotrichida and Heterotrichida.

In rhizopods the roving vacuole adds to its volume by picking up fluid substances from all parts of the endoplasm until it becomes too heavy to be easily moved with the flowing endoplasm. The vacuole is thus gradually left behind, so to speak, until it finally breaks through the thinning wall of protoplasm and empties its contents to the outside, usually at that part of the body which for the time being is posterior. In the fixed forms of vacuoles the fluids to be excreted are brought to the excretory organoid by more or less definite routes or canals, through the endoplasm. Such canals are highly characteristic of many types of ciliates. A familiar example is afforded by the different species of *Paramecium* where the five or ten radiating canals form a characteristic rosette about each of the two contractile vacuoles (Fig. 85). In the Hypotrichida there are usually two such canals leading to the dorsally placed vacuole, and two in *Stentor*, one following the margin of the body to the "foot," the other following the rim of the peristome in a circular course around the body (Fig. 74). In *Ophryoglena flava* there may be as many as thirty fine feeding canals leading from all parts of the body to the centrally-placed vacuole and in *Frontonia leucas* eight to twelve such canals follows a tortuous course throughout the body substance. In *Pycnothrix* the canals form a branching network through the endoplasm. Such canals are replaced by a ring of feeding vacuoles in many of the corticate flagellates and Dinoflagellates.

In corticate Protozoa the contractile vacuole usually opens to the outside in the vicinity of the anus when such a structure is present. In many cases it opens into the cytopharynx as in the majority of flagellates or in the vestibule of forms like *Vorticella*.

In *Euglena* and its allies the fluids of the contractile vacuole do not pass directly to the outside but are stored for a longer or shorter period in reservoirs which thus function like a bladder. In *Cam-*

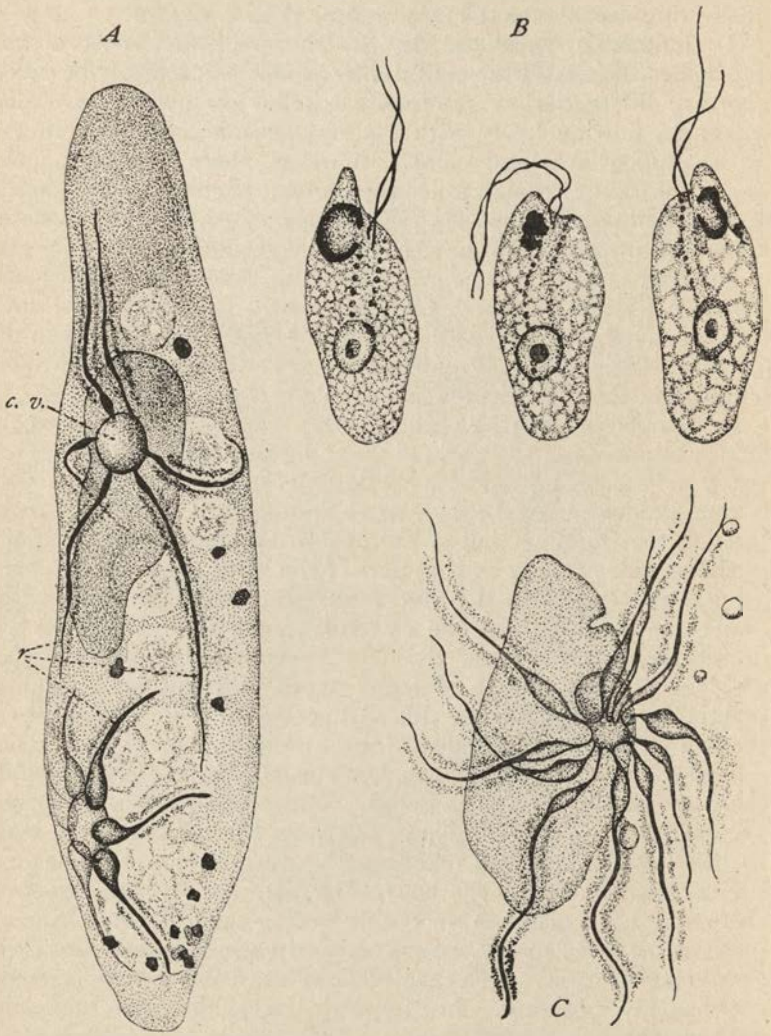

FIG. 85.—Golgi bodies in *Chilomonas paramecium* (*B*) and *Paramecium caudatum* (*A* and *C*). *c.v.*, Contractile vacuole; *r*, radial canals of *Paramecium*. (After Nassonov.)

panella umbellata such a reservoir is replaced by two definitely walled evacuation canals, while in *Pycnothrix* the excretory canal is said to be provided with special cilia.

In Dinoflagellata, according to Schütt (1892), there are, in addition to the contractile vacuole, large fluid-filled vacuoles which likewise open to the outside at the flagellum fissure by a fine pore canal. These are termed sac-pusules by Schütt and differ in function apparently from the collecting pusules or contractile vacuoles proper. Doflein suggests that these larger vacuoles may perform a hydrostatic function. The term pusule is applied by Schütt in view of the presence of distinct membranes and the absence of rhythmical pulsations, features which distinguish them from ordinary contractile vacuoles.

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CHAPTER IV.

GENERAL PHYSIOLOGY.

LIFE, ORGANIZATION, AND VITALITY.

There is no doubt that our knowledge of the structures of Protozoa far outstrips our knowledge of their functions. The minute size of the individuals and the inadequacy of microchemical tests make it extremely difficult to follow out any physiological process to its end, and masses of single cells in pure culture are impossible to obtain, although an approach in this direction is made by the so-called "pure-mixed" cultures of Bacteria and Protozoa.

It must not be overlooked that physiological problems here for the most part begin where similar problems of the Metazoa leave off, namely in the ultimate processes of the single cell. Here the functional activities have to do with the action and interaction of different substances which enter into the make-up of protoplasm and, at the present time, are beyond our powers of analysis. A few of these activities may be duplicated individually and apart from correlated functions, in the laboratory. Or specific reactions between specific chemical substances may be obtained as, for example, the digestion of fibrin by fluids extracted from the protozoön protoplasm; or in a physical sense the reversal of the sol and gel states in colloidal mixtures. Such individualized processes, however, give little idea of the infinite play of forces continually operating in living protoplasm all of which, harmoniously working together, make up the phenomena of vitality and distinguish living from lifeless matter.

Protoplasm is an aggregate of chemical substances in colloidal form and in the physical state of a complex emulsion. Groups of chemical substances known as nucleins, nucleo-proteins, albumins, carbohydrates, lipoids, salts and water are universally present and the various vital activities consist of actions, reactions, and interactions between and amongst these different substances. Aggregates of substances become local centers of special activity; these, the plastids of the cell enumerated in Chapter II, frequently have a definite form and size and can be demonstrated morphologically. Such plastids do not lose their lability or functional activity with the processes in which they participate. Other substances, however, are chemically and physically changed by the processes through

which they pass and become stabile elements of the protoplasmic make-up. Such changed substances no longer enter into the vortex of vital activities, but as metaplastids may or may not be of further use to the organism.

The almost infinite variety of form and structure represented by the Protozoa in the last analysis, must be traced back to the chemical nature of the proteins and to their relations and interactions with other substances in protoplasm. Types which have a similar chemical and physical make-up, with similar metaplastids and plastids, are practically identical in form and structure and we recognize them as distinct species. Variations in chemical composition, be they ever so little, must result in different chemical reactions and products with corresponding variations in form and structure of the organism, and these variations furnish the basis for classification.

Under normal conditions the reactions amongst the varied substances in protoplasm of the same species, with their products and arrangement of these products, are individual and invariable. Furthermore, the entire organism partakes of this individuality. A fragment of *Stentor* obtained by cutting or by shaking cannot be distinguished from a similar fragment of *Dileptus*, yet the former regenerates into a perfect *Stentor*, the latter into a perfect *Dileptus*. Or an encysted *Uroleptus mobilis* is morphologically identical with an encysted *Didinium nasutum;* both are apparently homogeneous balls of undifferentiated protoplasm; the one emerges from the cyst with the characteristic differentiations of *Uroleptus*, the other of *Didinium*. In short, the homogeneous ball representing *Uroleptus* is as specific and different from the homogeneous ball representing *Didinium*, as the adult *Uroleptus* is different from the adult *Didinium*. We may speak of this specific chemical and physical make-up as the *fundamental organization* of the species, or of the specific protoplasm, in a sense similar to the architectonik of Driesch. The adult characteristics are the outcome of the specific chemical make-up of the proteins, carbohydrates, salt, water, etc., and of the interactions amongst them and represent what we may call the *derived organization.*

Organization in the above sense is not only specific but continuous from generation to generation, and has come down through the ages subject, however, to modifications and changes through interaction with the environment or through changes coming from within as in amphimixis.

While organization is continuous the actions and reactions going on within it are discontinuous. More or less prolonged periods of rest are characteristic of all living things, best exemplified in the case of spores, eggs, encysted Protozoa and seeds. At such times the organization is static; the chemical substances making up the

specific organization are present but quiescent, or at most relatively inactive. A striking illustration is afforded by the phenomenon of desiccation in some types of animals, *e.g.*, rotifers. For some years I had on my shelf a bottle of minute amorphous granules which appeared like specks of dust under the microscope. After placing a few of these granules in water each of them would become an active, living rotifer in an hour or so. Here organization was present but inactive and activity began with the absorption of water and with oxidation. The rotifer in the active state is the same rotifer that it was in the dried condition, so far as organization is concerned, but it differs in that the organization is now in action. It is a difference of the same nature as that between an automobile standing in the garage, and the same automobile travelling 30 miles an hour. The organization is in action in both moving rotifer and moving automobile; is static in the dried rotifer and in the standing machine.

For descriptive purposes, at least, we find a decided advantage in a clear discrimination between these two states of living matter, viz.: organization in the quiescent or resting condition, and the same organization in action. We would limit use of the term *Vitality* to the active state and would define it as the sum total of actions, reactions, and interactions going on between and amongst the substances making up protoplasm and between these and the environment. The concept *Life*, with its attribute of continuity, thus becomes associated with the concept of organization rather than with the more dynamic concept of activity, which is intermittent or discontinuous. Life cannot be defined or measured any more than we can define and measure organization. Vitality, on the other hand, can be measured both as a whole and in its constituent activities.

As a result of activities the protoplasmic organization itself may change. An encysted *Uroleptus* is a motionless and apparently a homogeneous ball of protoplasm; an hour later it is an elongate, cigar-shaped organism with specialized motile organs in the form of cilia, membranelles and cirri, and its contractile vacuole pulsates with rhythmical regularity as it moves actively about in the water. The organization has undergone a change in this brief period; the first indication is the swelling and enlargement of the cyst wall, evidently by the absorption of water; oxidation probably occurs and substances already present, or new substances formed as a result of this initial oxidation, are responsible for the newly-developed structures or derived organization not present before. Such structures, however, are the morphological expression of the adult organization and their formation corresponds to development and differentiation of the metazoön egg.

Continued activity involves other and still more subtle changes in

organization; some of these are evident in individual life between division periods; others are evident only in a long series of individuals constituting a life cycle. These will be more fully treated in Chapters X and XI.

Other changes in organization may be brought about by environmental conditions; or they may be brought about by changes in one or more of the substances constituting the protoplasm of the species, as when amphimixis introduces a new combination of chromatin into the organization. These are undoubted factors in the phenomena of adaptation and probably play a part in the origination of new species and types.

In presenting the physiological aspects of the Protozoa in this and in the later chapters, I shall endeavor to follow out the train of thought outlined above. The more obvious functional activities of the individual will be considered first, and will be followed by a discussion of vitality or the sum total of activities in the life cycle and the changes in organization which accompany the changes in vitality. Sex phenomena, including gamete formation and maturation, will be treated as evidences of cyclical differentiation in the organization, while fertilization phenomena will be considered from the standpoints of their bearing on reorganization and vitality and on the origin of specific variations.

FUNCTIONAL ACTIVITIES OF THE INDIVIDUAL.

The sum total of the various physiological processes of the individual may be subdivided for the Protozoa, as they are for the Metazoa, into aggregates of special activities which we call the fundamental vital functions, and distinguish as nutrition, excretion, irritability and reproduction. In Metazoa these are performed by specialized cells, grouped into tissues, organs and organ systems, the complexity varying with the specialization of the organism. In Protozoa they are all performed by the single cell and all are more or less involved in the activities of the diverse substances and structures which compose it. All work together in a harmonious cycle of matter and energy.

The scientific beginnings of the modern mechanistic conception of vital activities is traced to Lavoisier and his comparison of animal heat with physical heat due to combustion through oxidation. The utilization of chemical energy or energy of combination liberated by oxidation, is possibly the keynote to the multiple vital harmonies of animal life (see Verworn, 1907). Oxygen necessary for such physiological combustion is obtained by all protozoa without the aid of specialized respiratory organs. It is readily absorbed through permeable membranes from the surrounding water, or obtained by reduction from oxygen-holding substances, as in anaërobic forms.

In one way or another it is ever present to initiate the round of vital functions.

The energy of combination, released by oxidation, is paid for by loss in the chemical compound oxidized. Other compounds may be formed with lessened energy of combination, and end-products, notably CO_2 and urea $(NH_2)_2CO$ are not only useless to the organism but positively harmful unless voided. Excretion, therefore, must follow oxidation. To make good the loss of substance new food materials must be taken in, digested and assimilated, but this is possible only through movement, and movement in turn is an expression of irritability. Hence a second consequence of oxidation is movement and the latter is made possible because of the energy transformed by oxidation. Excretion and irritability thus are fundamental vital functions, while a third, nutrition, is closely correlated. Excess of food intake over waste by oxidation leads to growth of the diverse protoplasmic substances and to their reduplication by division, while the aggregate of such divisions, expressed visibly by division of the cell, constitutes reproduction. The fundamental vital functions are thus intimately bound together; external conditions such as decrease in temperature of the medium in which a protozoön lives, means decreased oxidation, retarded movements, less food and a lower division rate. Increase in temperature involves a speeding up of all activities and, if food is abundant, a higher division rate. External conditions involving absence of food lead to starvation and death of the cell through uncompensated loss by oxidation. In short, interference with any one of the fundamental functions leads to disturbance of them all, and the various phases of vitality of the protoplasm during a typical life cycle may be due to inadequate functioning of one or another or all of these activities.

A. Excretion of Metabolic Waste.—The waste matters of oxidation and continued metabolism are frequently voided in the same manner that water and oxygen are taken in, namely, by osmosis. In such cases there is no physiological need of specialized excretory organs. It is possible that all Protozoa excrete in this way, although the majority of fresh-water Protozoa possess contractile vacuoles which are generally regarded as excretory organs. In marine forms and in parasites they are generally absent. If such forms, and these are the majority of Protozoa, are able to dispose of the products of destructive metabolism without definite organs for the purpose, why are the latter necessary in fresh-water forms? Hartog (1888) has long maintained that contractile vacuoles are not obligatory excretory organs, but are primarily hydrostatic organs for the purpose of maintaining a pressure equilibrium between the fluids within the cell and those in the surrounding water. Degen (1905) interprets the vacuole in a similar way, its variations in size and pulse depending upon permeability of the membrane which varies

with the environmental salts. If a given organism lives in a hypertonic medium, water formed as a waste product of metabolism does not accumulate but passes out by exosmosis as in marine forms. Some types of Protozoa, furthermore, may be transferred from fresh water to salt without fatal results. Thus Zuelzer (1903) has shown that *Amœba verrucosa* upon transference from fresh water to salt continues to live. It not only loses its contractile vacuole but the protoplasm becomes much condensed, evidently through loss of water. Here difference in density of the surrounding medium is largely responsible for loss of the organ characteristic of fresh-water forms, but changes in permeability of the cell membrane due to salts in the new medium undoubtedly play an important part. Other experiments by different observers bear out the same principle. Thus dilution of the normal neutral salts in the medium causes enlargement of the contractile vacuoles in *Euglena* and in ciliates according to Klebs (1893) and Massart (1891), while increased concentration leads to reduction in size, retardation in rate of contraction, or total disappearance of the vacuole.

While there is justification for Hartog's view of the purely physical significance of the vacuole, there is every reason for believing that water in protoplasm picks up any soluble waste matter that may be present, and holds it in solution. Early experiments to prove this, by Brandt (1885), Griffiths (1889), and others using chemical indicators, or the murexid test for uric acid, were not convincing, and the function of the contractile vacuole as a primitive type of excretory organ remained an hypothesis.

Not only water, CO_2 (see Lund, 1918) and urea, but other products of metabolism as well, are frequently found in the protoplasm of different Protozoa. These are usually present in crystalline form or in amorphous heaps, which are rather loosely spoken of as "excretory stuffs" without evidence as to their origin and significance. The crystals often seen in *Paramecium* were identified by Schewiakoff (1893) as calcium phosphate combined with some organic substance. Similar crystals have been described by Schaudinn, Schubotz and others from the protoplasm of different kinds of Protozoa. Schewiakoff found that the crystals of *Paramecium* are not defecated as are undigested food substances, but are first dissolved and then disposed of—presumably with the water of the contractile vacuoles.

The function of the contractile vacuole in Protozoa thus has long been a disputed problem. The views of the older students of the group, with their conceptions of structural complexity of these unicellular organisms, fantastic today, nevertheless have a certain historical interest. The idea that a vacuole is a rudimentary beating heart as interpreted by Lieberkühn (1856), Claparede and Lachmann (1854 and 1859), Siebold (1854) and Pritchard (1861) was no less

incongruous than the supposition of Ehrenberg (1838) that the contractile vacuole is an organ connected with the gonadal system.

With development of knowledge of structure and function of the Protozoa, and particularly of the mechanism of vitality, more reasonable hypotheses of the function of the contractile vacuole have been developed. There is, first, some ground for the belief of Spallanzani (1776), Rossbach (1874) and Dujardin (1841) that it is an organoid having to do with respiration of the organism, together with other possible functions, a view supported in modern times by Bütschli (1877, 1888) and Degen (1905). There is, second, ground for the belief held by Stein (1859), Gruber (1889) and the majority of modern students of Protozoa, that it is an organoid for the excretion of katabolic waste, despite the unconvincing experimental evidence by Brandt (1885), and by Griffith (1889). Howland (1924), however, by using a much more delicate test (the Benedict blood-filtrate test) obtained unmistakable evidence of the presence of uric acid in Protozoa; in *P. caudatum* analyzed by Benedict, a color reaction was obtained equivalent to 4 to 5 mg. of uric acid per liter. There is, third, ground for the belief that the contractile vacuole is an organoid for the regulation of osmotic pressure in the cell, a view first advanced by Hartog (1888) and supported by Degen (1905), Stempell (1914), Khainsky (1910) and recently by Nassonov (1924).

These three beliefs are not necessarily exclusive and the possibility of all three functions is still open. The osmotic function is well supported by evidence furnished by Gruber's (1889) experiments in transferring fresh-water vacuole-holding *Actinophrys sol* and *Amœba crystalligera*, to salt water, and *vice versa*, or by Zuelzer's similar experiment with *Amœba verrucosa*, the protoplasm becoming more condensed and the vacuole lost in salt water. Hogue (1923) found that *Vahlkampfia calkensi* when transferred from salt water to fresh-water media developed 1, 2, 3, or even 4 contractile vacuoles. More extensive experiments by Degen (1905) with salts of different kinds and with varied conditions of the environment, show that the contraction of the vacuole is a function of osmotic pressure, and irrespective of the type of salt or neutral solution introduced. With Hartog, he concludes that protoplasm of fresh-water forms, with its salts in solution, has a higher osmotic pressure than the surrounding medium, which leads to continued intake of water. Such intake, if not balanced, would lead to inflation and to diffluence. According to Degen and Hartog it is the function of the contractile vacuole to establish this balance.

This hypothesis, with further evidence supplied by the absence of contractile vacuoles in marine forms where osmotic relations of protoplasm and environment are more evenly balanced, is theoretically correct. There is no reason to doubt, however, the further possibility that the water expelled by the contraction of the vacuole

contains water-soluble katabolic excretory substances such as CO_2 and nitrogenous waste, positive evidence for which is supplied by Howland. This indeed was admitted by Degen although he obtained no evidence of the nature of the substances excreted. He saw in the membrane of the vacuole the possibility of an excretory mechanism. The actual existence of such a membrane, however, is still in dispute, indeed the majority of investigators deny its existence (Bütschli, Rhumbler, Schewiakoff, Taylor). Others, however, give evidence to show that a true membrane, although very delicate, is actually present. Howland (1924, 1) for example, by micro-dissection methods has been able to remove the contractile vacuoles of *Amœba verrucosa* and of *Paramecium caudatum* after which they retain their integrity for considerable periods as free vacuoles in the surrounding water. She also has punctured the vacuole with needles while in the endoplasm, causing the expulsion of its contents into the surrounding endoplasm and resulting in the wrinkling of the vacuole membrane. Nassonov (1924) also not only demonstrates the presence of a membrane in various types (*Paramecium caudatum*, *Lionotus folium*, *Nassula lateritia*, *Campanella umbellaria*, *Vorticellidæ*, and *Chilomonas paramecium*) but by use of fixation methods employed for demonstrating the Golgi apparatus in metazoan cells, comes to the conclusion that the membrane of the contractile vacuole is the homologue of the Golgi apparatus. This, in Metazoa, he had earlier (Nassonov, 1923) identified as an organoid intimately bound up with secretory activities of the cell (see also Bowen). In different Protozoa the contractile vacuole, which he unhesitatingly calls an excretory apparatus with a definite lipoid membrane, is variously complicated, from a simple vesicle with osmiophilic membrane in forms like *Chilomonas paramecium* (Fig. 85, *B*), to complex aggregations of vesicle and canals as in *Paramecium* (Fig. 85, *A*, *C*). In the latter case the canals appear to contain the material by activity of which substances are chemically differentiated for secretion and these are passed on to the vesicle through whose activity they are excreted. With this work of Nassonov's, which is convincingly presented, we have a very definite statement of the excretory functions of the contractile vacuole and of the presence and function of the lipoid membrane. In quite a modern way it brings us dangerously near to an Ehrenbergian conception of a kidney and bladder in Protozoa.

B. Irritability.—In the absence of all knowledge as to the manner in which protoplasmic particles respond to stimuli of different kinds, we are constrained in speaking of irritability of Protozoa, to limit descriptions to aggregates of such responses as manifested through movement, as energy transformed by oxidation from the potential or stored chemical energy, to the active of kinetic condition. But the manner in which such kinetic energy is utilized in pseudo-

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contains water-soluble katabolic excretory substances such as CO_2 and nitrogenous waste, positive evidence for which is supplied by Howland. This indeed was admitted by Degen although he obtained no evidence of the nature of the substances excreted. He saw in the membrane of the vacuole the possibility of an excretory mechanism. The actual existence of such a membrane, however, is still in dispute, indeed the majority of investigators deny its existence (Bütschli, Rhumbler, Schewiakoff, Taylor). Others, however, give evidence to show that a true membrane, although very delicate, is actually present. Howland (1924, 1) for example, by micro-dissection methods has been able to remove the contractile vacuoles of *Amœba verrucosa* and of *Paramecium caudatum* after which they retain their integrity for considerable periods as free vacuoles in the surrounding water. She also has punctured the vacuole with needles while in the endoplasm, causing the expulsion of its contents into the surrounding endoplasm and resulting in the wrinkling of the vacuole membrane. Nassonov (1924) also not only demonstrates the presence of a membrane in various types (*Paramecium caudatum*, *Lionotus folium*, *Nassula lateritia*, *Campanella umbellaria*, *Vorticellidæ*, and *Chilomonas paramecium*) but by use of fixation methods employed for demonstrating the Golgi apparatus in metazoan cells, comes to the conclusion that the membrane of the contractile vacuole is the homologue of the Golgi apparatus. This, in Metazoa, he had earlier (Nassonov, 1923) identified as an organoid intimately bound up with secretory activities of the cell (see also Bowen). In different Protozoa the contractile vacuole, which he unhesitatingly calls an excretory apparatus with a definite lipoid membrane, is variously complicated, from a simple vesicle with osmiophilic membrane in forms like *Chilomonas paramecium* (Fig. 85, *B*), to complex aggregations of vesicle and canals as in *Paramecium* (Fig. 85, *A*, *C*). In the latter case the canals appear to contain the material by activity of which substances are chemically differentiated for secretion and these are passed on to the vesicle through whose activity they are excreted. With this work of Nassonov's, which is convincingly presented, we have a very definite statement of the excretory functions of the contractile vacuole and of the presence and function of the lipoid membrane. In quite a modern way it brings us dangerously near to an Ehrenbergian conception of a kidney and bladder in Protozoa.

B. Irritability.—In the absence of all knowledge as to the manner in which protoplasmic particles respond to stimuli of different kinds, we are constrained in speaking of irritability of Protozoa, to limit descriptions to aggregates of such responses as manifested through movement, as energy transformed by oxidation from the potential or stored chemical energy, to the active of kinetic condition. But the manner in which such kinetic energy is utilized in pseudo-

podia formation or by the elements of flagellum, cilium or myoneme, is a matter of pure speculation. The reactions which characterize the resulting movements, however, can be analyzed and measured and these form the chief basis of our knowledge of protozoan irritability. Attempts to explain pseudopodia formation and amœboid movement have varied with the changes in our conceptions of the physical make-up of protoplasm. The protoplasm of *Amœba* regarded as a fluid substance, was supposed to follow the laws of surface tension characteristic of all fluids. Pseudopodia formation, according to the views of Berthold (1886) is the attempt of one fluid (protoplasm) to spread out between water and the substratum as Quincke's well-known experiments demonstrated for fluids. As physical conditions on all sides of the Amœba are not equal, variations in tension result in local diminution, and the tendency to spread is focused in a local area and the pseudopodium results. Bütschli's (1894) observations and experiments with emulsions of oil, salts and water, and Rhumbler's (1898) analysis of the causes of movement in lobose rhizopods led these observers also to interpret pseudopodia formation as a result of surface tension phenomena. With the more modern conception of protoplasm as a colloidal aggregate in the physical state of an emulsion in which the external and internal protoplasm of *Amœba* are in the relation of gel and sol, the difficulty of applying the laws of fluids became apparent and the hypothesis based upon surface tension has been generally abandoned. Rhumbler himself (1910 and 1914) recognized this difficulty and materially changed his conception of amœboid movement, while Hyman (1917) greatly enlarged and perfected his later point of view. According to Hyman the ectoplasm of *Amœba*, by virtue of its relatively solid state, becomes tenuous but elastic, as demonstrated by the experiments and observations of Jennings (1904), Kite (1913, Schultz (1915) and Chambers (1915, 1917), and exerts an elastic tension on the inner fluid protoplasm. Bancroft (1913) and Clowes (1916) demonstrated the reversibility of phase in diphasic physical systems through the agency of electrolytes, and the conclusion followed that the ectoplasm represents a reversal phase of the more fluid inner protoplasm. Hyman argues that, owing to the tension of the enveloping ectoplasm, if any local region of the solid ectoplasm becomes liquefied, the resistance gives way at such a point and the fluid endoplasm is pressed out, thus forming a pseudopodium. The immediate cause of such liquefaction she traces to a local increase of, or change in, metabolic activity resulting in the production of hydrogen-ions appropriate for dissolution of the solid ectoplasm. By use of Child's potassium cyanide test for metabolic gradients, she was able to demonstrate that such local regions of greater metabolic activity actually occur on the periphery of *Amœba proteus* before a pseudopodium breaks out, also

that the extreme tip of the advancing pseudopodium is the most actively metabolic part.

On the basis of some such physical interpretation of amœboid movement, the problem of harmonizing pseudopodium formation with the activities of flagella, cilia and ciliary aggregates, does not appear as hopeless as it does upon the surface tension hypothesis. Elasticity of *Amœba* ectoplasm and of endoplasmic solidification (stereome) in Foraminifera, elasticity and contractility of axial filaments in Heliozoa or in axial fibrils (kinetic) of flagellates and ciliates, may ultimately be harmonized on the basis of some physical explanation of this nature.

Whether repeated shocks leading to changes in the nature of protoplasmic response or to changes in direction of movement should be interpreted on the basis of "memory" and "learning" or in some other way is largely a matter of personal idiosyncrasy on the part of the observer. Numerous observers have described processes of food "selection" by *Amœba* (*e. g.*, Gibbs and Dellinger, 1908; Schaeffer, 1917 and elsewhere, Metalnikoff, 1910, *et. al.*). Mast and Pusch (1924) interpret an observed change in the protrusion of pseudopodia of *Amœba proteus* in respect to a beam of light as evidence of something analogous to "learning" in higher animals, etc. "Learning" involves "memory," and such terms connote processes of an entirely different nature which we associate with the highest types of animals. It is conceivable that fatigue, to use the term in its broad sense implying total or partial exhaustion of protoplasmic constituents necessary for a reaction, and therefore a purely physical matter, is adequate for explanation without calling upon any obscure pan-psychic analogy. Similarly with Kepner and Taliaferro's (1913) evidence of "purpose" in methods of food-getting by *Amœba proteus*.

Many of the reactions of Protozoa are bound up with the coördinating mechanism of the cell through which the organism acts as a unit. The specific response of an organism to a stimulus is the result of its particular protoplasmic architecture expressed through its coördinating mechanism and motile organs. This has been elaborately worked out by Jennings (1904 to 1909) in connection with the "motor response" of many different kinds of Protozoa.

The discussions and controversies over the matter of directive stimuli or tropisms in Protozoa have evidently been due in large part to a lack of a common understanding of the definition. If by "tropism" is meant the orientation of an organism in respect to the path of a stimulus, then tropisms, as Jennings was the first to point out, play little part in the activities of the Protozoa. If, however, by "tropism" is meant "the direct motor response of an animal to an external stimulus" (Washburn, 1908), then tropisms play a most important part in such activities. The two definitions are not

compatible; the former conveys the idea of a directive stimulation upon local motor organs or controlling elements; the latter implies the complex reaction of a definite mechanism characteristic of any specific protoplasm, and the same reaction follows upon stimulation by any type of stimulus (Pütter, 1903, Jennings, 1909). It follows further that the reaction is called forth regardless of the particular organ or element first to receive the stimulus.

We owe Jennings the credit for first clearly distinguishing between these two conceptions, as well as for careful analyses of the movements of lower organisms (1904 *et. seq.*), and for demonstrating the particular motor response distinctive of specific types of Protozoa. He also showed that the nature of the motor response in some organisms, *e. g.*, in *Stentor*, is correlated with the physiological state of the organism, and adduced evidence which indicates that phenomena of fatigue are involved. The classical example of a motor response, formerly interpreted as chemiotaxis, is the case of *Paramecium caudatum* or *aurelia* in a drop of dilute acid. Casual swimming brings the individual to the outer limit of the drop; the transition from water to drop does not provide a stimulus strong enough to bring about the motor response and the individual continues through the drop until it strikes the farther limit. Here the stimulus is sufficiently strong to cause the motor response which is manifested as a backward swimming, due to reversal of cilia, turning on the long axis and recovery of normal forward swimming movement. Repetition of this procedure keeps the individual in the acid drop. Others enter in a similar way and are similarly trapped until many are confined in the acid drop where they are ultimately killed. Such motor responses unquestionably play an important role in food-getting and in vital activities generally.

The stereotyped nature of the motor response in any specific organism may be due in the main to the characteristic neuromotor systems which the higher types of flagellates and ciliates possess. The observations of Sharp (1914), Yocom (1916) and McDonald (1922) on ciliates, of Kofoid on flagellates, and the experiments of Taylor (1920) in cutting different regions of the neuromotor complex of *Euplotes*, indicate that the motor response of Protozoa is bound up with coördinating systems possessing some of the attributes of coördinating systems in Metazoa (Fig. 86). Knowledge of these complex systems and their reactions is quite sufficient to dispel any lingering belief in tropisms as due to stimulation of special motile elements acting independently in such a way as to orient the organism in respect to the path of the stimulus. Through coördinating fibrils all parts work together; cutting the system at any point leads to inharmonious or uncoördinated movements of the motile organs as Taylor has demonstrated. All reactions depend upon the organism as a whole; enucleated fragments are unable to

react as do nucleated fragments (Hofer, 1890, Willis, 1916). Jennings' careful observations, which led him to the conclusion that the protozoön organism always acts as a whole is fully confirmed by these later observations and experiments.*

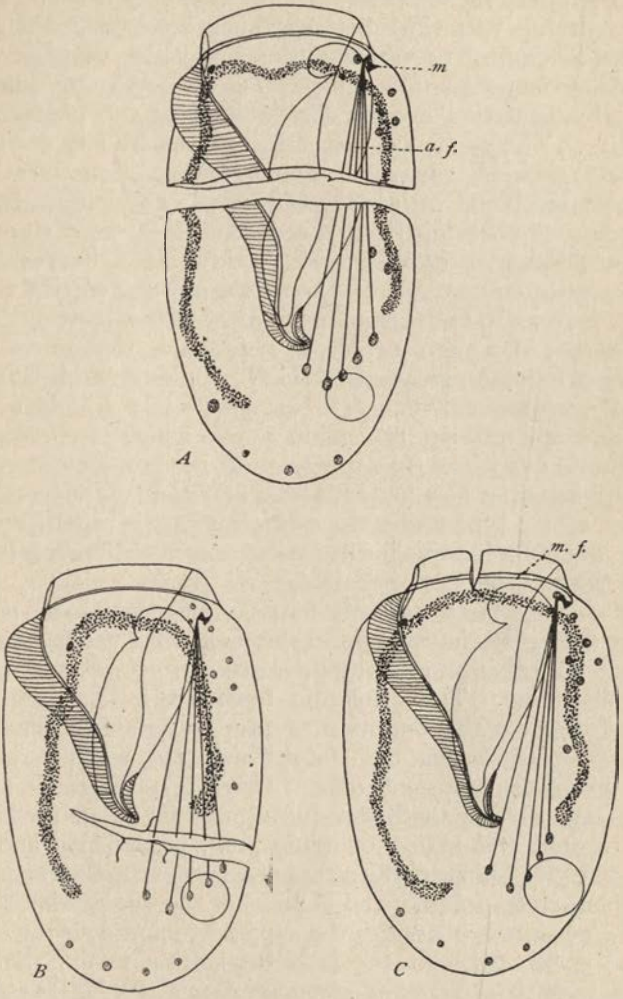

FIG. 86.—Merotomy in *Euplotes patella*. (After Taylor.) *a.f.*, anal cirri fibers; *m.*, motorium; *m.f.*, membranelle fiber. (See also Fig. 57.)

C. Nutrition.—Under the heading nutrition are included all physiological processes involved in the replacing of substances

* For discussion of different types of stimuli and the resulting reactions by Protozoa see Minchin (1912), Khainsky (1910), Mast (1910–1918), Pütter (1900, 1903), Jennings (1904, 1909).

exhausted by destructive metabolism. Groups of activities including: (1) Food-getting; (2) digestion and secretion; (3) assimilation; (4) defecation, find their place here. The specialized structures adapted for these various activities have been described for the most part in the preceding chapters, and the following is supplementary in nature dealing with the functions which these structures perform.

1. **Food-getting.**—The varied methods by which Protozoa acquire the needed materials for replenishing protoplasmic substances reduced by oxidation are all correlated with the phenomena of irritability. The particular method employed by any one type of organism is probably the result of many factors of organization and adaptation combined with mode of life, all of which are traceable to adaptations resulting from the effects of external stimuli and response through irritability. It would indeed be remarkable, considering the endless variety of endoplasmic and cortical differentiations, were we to find a common method of food-getting amongst the Protozoa. On the contrary, it is probable that no two types of organism follow an identical method. Nevertheless it is possible, and it is certainly convenient, to group these manifold activities under a comparatively few main types which are designated: (1) Holozoic nutrition; (2) saprozoic nutrition; (3) autotrophic or holophytic nutrition; (4) heterotrophic nutrition. Many authorities introduce a fifth type under the caption parasitic nutrition, but as this does not differ in principle from saprozoic nutrition, it is included with the latter type.

While these terms apparently indicate different modes of nutrition, the differences have to do in the main with the nature of the raw materials taken in and the subsequent processes necessary for their elaboration. Thus holozoic nutrition in Protozoa as in Metazoa involves the ingestion of raw materials in the form of proteins, carbohydrates and fats which are usually combined in the protoplasm of some other living organism eaten as food. It is an expensive method of acquiring raw materials for it necessitates capture and killing of living prey, preparation and secretion of digestive fluids and ferments necessary to make the proteins and carbohydrates soluble, and disposal of the undigestible residue. On the other hand, it assures the supply of capital in the form of chemical energy without the labor of storing it up. Saprozoic nutrition is, so to speak, a more economical method, for the organism does away with the elaborate processes of secretion and digestion and relies upon the activities of other organisms for the preparation of its raw materials and the "storage of energy." Dissolved proteins and carbohydrates made soluble through the agency of bacteria and other organisms in infusions, or prepared by the digestive processes of the host in the case of parasites and some commensals, are absorbed directly through the body wall or through

special receptive regions, by endosmosis. This type of food-getting may be regarded as a degeneration or adaptation of the holozoic method, the specialized absorptive areas being reminiscent of former mouths, while the pathogenic effects of some types of parasites are interpreted as due to the secretion by the parasite of digestive fluids which cause cytolysis of the host cells. Holophytic or autotrophic nutrition, characteristic of the green plants, is quite different in principle from the other two. Digestive processes typical of the majority of animals, as well as the intake of solid or dissolved food, are absent. A highly labile substance, chlorophyll, is manufactured in the presence of light and usually by specialized plastids—chromoplastids—of the cell. Chlorophyll is very sensitive to light and in some way not yet understood is instrumental in utilizing the radiant energy of the sun to form complex, energy-holding compounds. Plants thus become the great banking house for animals and their capital is the apparently inexhaustible energy of the sun. Only those Protozoa with chlorophyll, standing on the boundary line between plants and animals, have this power to directly untilize the sun's energy (see infra p. 197). Heterotrophic nutrition, finally, is characteristic of those Protozoa which combine any two of the above methods of acquiring raw materials. Some forms combine holozoic with saprozoic methods; others holozoic and holophytic; others saprophytic and holophytic, and some combine all three.

(*a*) *Holozoic Nutrition.*—The great majority of Protozoa are holozoic in their methods of food-getting, and we may distinguish two main groups, the continuous feeders, and the occasional feeders. Continuous feeders are those forms with permanently open mouths through which a constant current of water is maintained by action of the peristomial motile apparatus (see p. 147). Minute forms of life, especially Bacteria, are carried by these currents into the endoplasm where they undergo digestion in improvised stomachs or gastric vacuoles (see p. 187). The majority of ciliates belong in this group including many of the holotrichous and all of the hypotrichous, heterotrichous and peritrichous ciliates.

The occasional feeders, like carnivorous types of Metazoa, feed whenever chance brings prey within the radius of their activity, and many of them, like cannibals, are guilty of feeding at times upon their close relatives (Maupas, 1883, Joukowsky 1898, Dawson 1919, Lapage 1922). In some cases balloon-like membranes are unfolded and spread out like sails for the direction of food currents to the mouth as in *Pleuronema chrysalis*. Such forms are intermediate between the constant and occasional feeding types. In other cases great net-like traps are spread for the capture of unwary diatoms, desmids or smaller Protozoa, as in the Foraminifera (Fig. 87). In other cases the microscopic hunters, like men in shooting boxes, lie in wait for their prey. Here long tentacles usually

radiate out from the body in the surrounding water as in *Actinobolus radians* or in Suctoria, until a victim comes in contact with one or more of the outstretched processes (Fig. 81, p. 154); in the same way axopodia of the Heliozoa capture chance organisms which serve as food (Fig. 88).

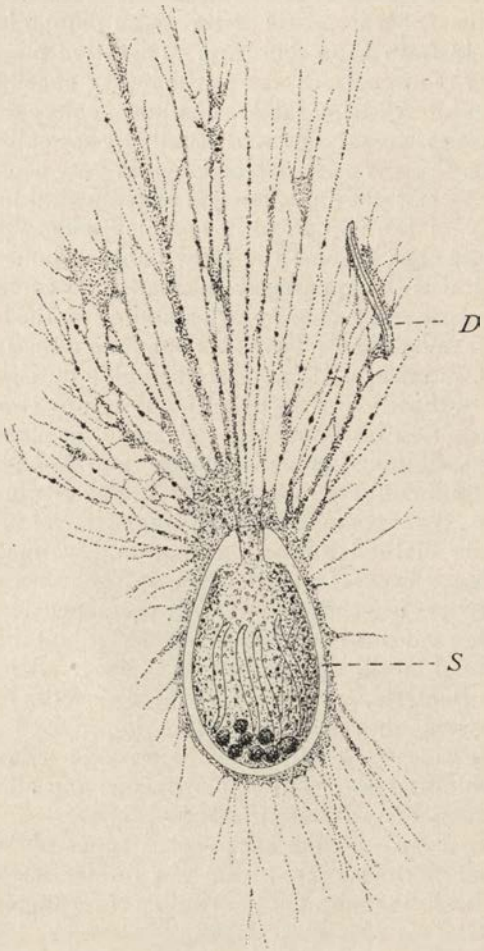

FIG. 87.—*Allogromia oviforme*, foraminiferon with chitinous monothalamous shell and reticulose pseudopodia. (*D*) a recently captured diatom; (*S*) chitinous shell. (From Calkins after M. Schultze.)

The most interesting of these holozoic types are the predatory forms which hunt their prey and capture them, while in full motion. The small, but powerful ciliate, *Didinium nasutum* belongs in this group. It darts here and there with an eccentric movement

while rotating at the same time on its long axis. In these sudden darts, it strikes a *Paramecium* or other ciliate purely at random, the proboscis with seizing organ is buried in the victim which is then swallowed whole (Figs. 88, *C*, and 89, 1–6). *Lionotus fasciola*, *Spathidium spathula* and other gymnostomatous ciliates capture living organisms in a similar way (Fig. 90) while less spectacular methods are employed by *Frontonia leucas*, *Ophryoglena flava*, *Prorodon niveus*, etc., in swallowing diatoms, desmids and other relatively stationary organisms.

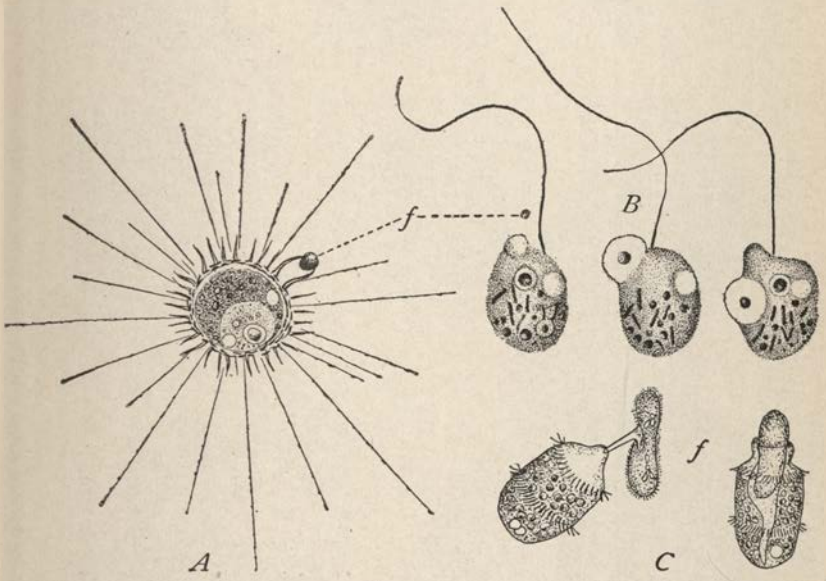

FIG. 88.—Types of food getting. *A*, *Raphidiophrys elegans* (after Penard); *B*, *Oicomonas termo* (after Bütschli); *C*, *Didinium nasutum* (after Bütschli); *f*, Food particles; in *C*, *Paramecium* is captured and eaten.

A special type of food-getting, illustrated by the Rhizopoda, may be interpreted in some cases as the result of physical properties of semifluid bodies. Rhumbler has made the most exhaustive studies of food ingestion in these forms and distinguishes four types, viz: Ingestion by (1) "circumvallation," (2) "circumfluence," (3) "invagination" and (4) "importation." Food taking by "circumvallation" is illustrated by *Amœba proteus* and usually takes place at that portion of the body which, for the time being, is posterior. According to Hofer (1889), Schaeffer (1917) and others, the body becomes anchored to the substratum by the secretion of an ectoplasmic gelatinous substance; then, through the physical stimulus (Schaeffer, 1917) produced by a moving object (even a moving needle point according to Verworn 1889), walls of protoplasm flow out on either

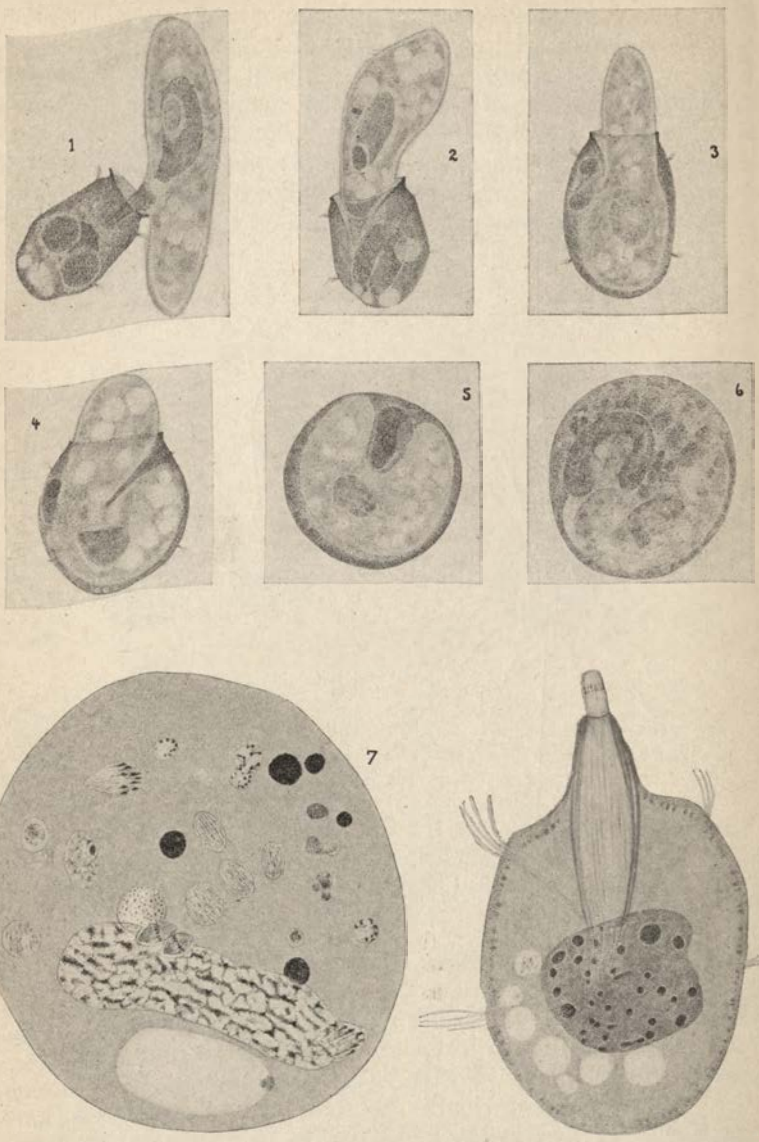

FIG. 89.—*Didinium nasutum* O. F. M. capturing and swallowing *Paramecium caudatum*. 1 to 6, Successive stages in the ingestion of *Paramecium;* 7, section of conjugating form of *Didinium* with spindle-form gastric vacuoles (?), and two micronuclei in mitosis; 8, section of *Didinium* just prior to encystment. The seizing organ with zone of trichocysts is protruded from the mouth; and rhizoplasts run from the membranulæ (motile organs) deeply into the cell. (After Calkins.)

side of the object and meet around it, thus enclosing a rotifer, an *Arcella*, a diatom or other food body. Ingestion by "circumfluence" appears to be due to a stimulus emanating from a living food body, the effect of which through the motor response (Jennings, 1904) is to cause pseudopodia to flow toward the prey and to entrap it while still at some distance from the body of the captor as in the testate rhizopods and Foraminifera. "Invagination" occurs in forms having a somewhat resisting periplast-like ectoplasm such as

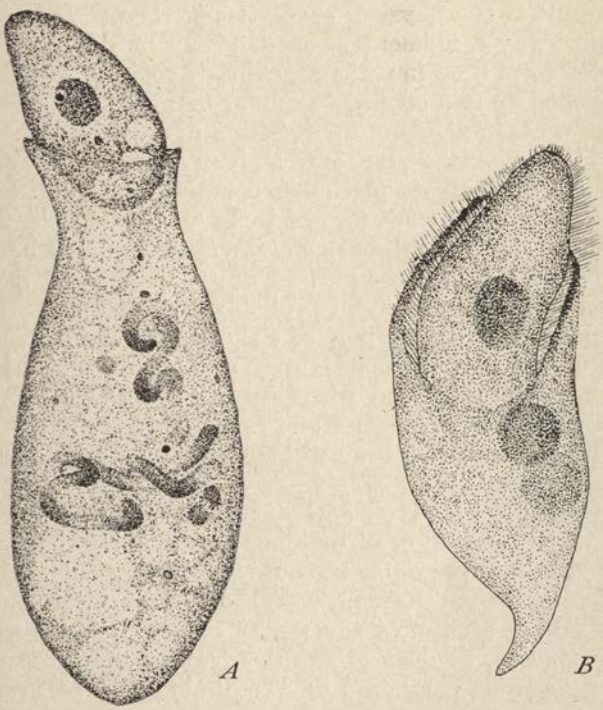

FIG. 90.—Two types of ciliated carnivores. *A*, *Spathidium spathula* about to ingest a *Colpidium colpoda; B*, *Lionotus fasciola* swallowing a *Colpidium colpoda.* (Original.)

Amœba terricola according to Grosse-Allermann (1909). When a living organism comes in contact with the surface at any point, the local ectoplasm with prey attached sinks into the endoplasm as though "sucked" in, the ectoplasmic walls being transformed into endoplasm, while the ectoplasm about the area of ingestion comes together sphincter-like, and fuses again to a smooth surface. So, too, in *A. proteus* where, according to Mast (1916 and 1923) and Beers (1924) the sphincter-like ingesting area is powerful enough to cut in two organisms like *Paramecium* and *Frontonia.* Ingestion

by "importation" finally occurs when a food body, without apparent movement on the part of the *Amœba*, merely sinks into the protoplasm of the captor as in *Amœba dofleini* according to Neresheimer.

In most of these types, which grade more or less into one another, the process of food ingestion may be interpreted as due to local liquefaction in the more solid ectoplasm, and to special conditions of capillarity in the more fluid endoplasm. Rhumbler has shown that a filament of Oscillaria which enters *Amœba verrucosa* by "importation" and is too long to be entirely engulfed, becomes coiled up as a result of the physical properties of the protoplasmic mass. In a similar way a filament of shellac may be drawn from water into a chloroform drop in which, by variations in surface tension, it becomes rolled up in a strikingly similar manner.

Some of these methods of food-getting in holozoic types are suggestive of "conscious" activities to a given end. Thus ingestion by "circumfluence" suggests preliminary activities in anticipation of a "square meal." Or traps formed by pseudopodia or by tentacles, or the balloon sails of *Pleuronema chrysalis*, etc., might be regarded as "set" by Protozoa for the purpose of catching food. Such interpretations, however, are more probably evidences of a temperamental imagination on the part of the observer than of purposeful activities on the part of these minute organisms. "Sensing" at a distance has been described for *Amœba* (Schaeffer, 1912), and for *Spathidium spathula* (Woodruff and Spencer, 1922), and until these phenomena are explained they will continue to serve as a basis for such speculations.

The so-called "selective" activities of some Protozoa in their apparent choice of food or of building materials for their shells are likewise better interpreted as the outcome of physical conditions of the protoplasm than as purposeful actions of the organisms. Schaeffer (1917) attributes the power of discrimination in food-taking to *Amœba*, as does Metalnikoff (1908), to *Paramecium*, a conclusion vigorously opposed by Wladimirsky (1916), who interprets negative reactions as a result of depression (fatigue?) of their physiological condition. *Actinobolus radians* apparently chooses, from a great number of miscellaneous forms, one particular species to harpoon, paralyze and swallow. "This remarkable organism possesses a coating of cilia and protractile tentacles which may be elongated to a length equal to three times the diameter of the body, or withdrawn completely into the body. The ends of the tentacles are loaded with trichocysts. When at rest the mouth is directed downward and the tentacles are stretched out in all directions, forming a forest of plasmic processes among which smaller ciliates, such as *Urocentrum turbo*, *Gastrostyla steinii*, etc., or flagellates of all kinds may become entangled without injury to themselves and without disturbing the *Actinobolus* or drawing out

its fatal darts. When, however, an *Halteria grandinella*, with its quick, jerky movements, approaches the spot, the carnivore is not so peaceful. The tentacles are shot out with unerring aim and the *Halteria* whirls around in a vigorous, but vain, effort to escape, then becomes quiet, with cilia outstretched, perfectly paralyzed. The tentacle with its prey fast attached is then slowly retracted until the victim is brought to the body and swallowed with one gulp. Within the short time of twenty minutes I have seen an *Actinobolus* thus capture and swallow not less than ten *Halterias*." (Calkins)

While these observations do not prove that *Actinobolus radians* eats nothing else, it is certainly true that the usual food is *Halteria grandinella*, a fact which may account for the rarity of *Actinobolus*. That it thrives on *Halteria* is proved by the fact that isolation cultures of *Actinobolus* have been maintained for a period of eight months and through 375+ generations by division during which the only food supplied was a daily ration of 2 to 3 dozen individuals of *Halteria grandinella* independent pure "mixed" cultures of which, with bacteria, were maintained at the same time. In these cases it is quite probable that the motor response due to some specific chemotactic stimulus is responsible for the apparent "choice" of food by *Actinobolus*, and chemotactic or thigmotactic stimuli for food capture by "circumfluence," "circumvallation" and "importation."

A certain degree of selection is forced upon some Protozoa by the limitations of their mouth parts. Forms like *Didinium*, *Spathidium*, *Lionotus*, etc., with distensible mouths, can handle organisms of various sizes, but forms like *Paramecium*, *Dileptus*, *Spirostomum*, etc., with small inelastic mouths are constrained to "select" small objects for food. Here there is no apparent choice between nutritious and innutritious particles, carmine or indigo granules being taken in with the same initial avidity as bacteria or other useful foods. A certain so-called "hunger-satisfaction," however, leads to the cessation of food-taking in many organisms. Thus *Actinobolus radians* often captures and paralyzes more *Halterias* than it actually eats; on one occasion, for example, an individual was seen to catch 18 *Halterias*, 11 of which were swallowed while a small group of 7 were abandoned uneaten, when the *Actinobolus* swam away.

Amœba proteus, after a period of eating no longer reacts to the stimulus of living food substances, and apparently ignores types which were previously engulfed (Schaeffer). So, too, in *Paramecium* and *Stentor*, Metalnikoff and Schaeffer describe an apparent selection of food as illustrated by the rejection of carmine granules after a period during which such granules were actually taken in. It seems probable that such phenomena indicate a type of fatigue involving the temporary loss of irritability through which the organism

responds to stimuli produced by the chemical make-up of foreign substances, a period of rest being necessary for the restoration of this form of irritability. Selection in another sense, however, is quite important. All kinds of food substances are not equally suitable for Protozoa any more than they are for individual men. This may be due to the fact that digestive fluids of a given type of ciliate or rhizopod are not adequate to dissolve all kinds of protein; or it may be due to deleterious substances in the protoplasm of the prey. All observers who have attempted to raise Protozoa in pure cultures are familiar with the difficulty of providing the proper food materials and excluding the harmful. Unsuccessful culture experiments indicate that these conditions have not been met. Furthermore, a culture medium is suitable only when the organism under cultivation continues to live during all phases of its life cycle. The failure of Calkins (1912) to rear a single exconjugant of *Blepharisma undulans*, or of Baitsell (1912) to raise exconjugants of *Stylonychia pustulata* are cases in point. The difficulties encountered in attempts to cultivate *Spirostomum ambiguum* or *Stentor coeruleus* are probably due to failure to find a suitable food or oxygen medium.* In some cases it is quite probable that a variety of proteins is necessary for the best cultural results. Hargitt and Fray (1917) and Phillips (1922) have shown that *Paramecium* will live on pure cultures of bacteria, but for active development they found that mixed pure cultures of certain types of bacteria give the best results.

Apparent selection of foreign objects used in shell building may be due to the physical consistency of the protoplasm and to its ability to pick up foreign bodies like sand crystals, diatom shells, etc., or in part to the size of the shell-opening through which such objects must pass for storage in the protoplasm. Mud and other fine particles of inorganic matter, like carmine granules, are engulfed with bacteria and other microörganisms which produce the stimulus necessary for the operation of food-taking. After the useful substances are digested the residue, like castings of worms, may be voided to the outside or they may serve a useful purpose in the construction of shells. Rhumbler (1898) was thus able to cause *Difflugia* to build its shell of finely-ground colored glass.

A special kind of holozoic food-getting is illustrated by the Suctoria which, instead of cilia, are provided with suctorial tentacles (Fig. 91). The prey, usually some form of ciliated Protozoa, comes in contact with one of these tentacles and is paralyzed through the action of some kind of poison contained in it. The cortex of the prey is perforated by the end of the tentacle and the fluid endoplasm is sucked into the body of the captor, a stream of granules being

* See note, page 25.

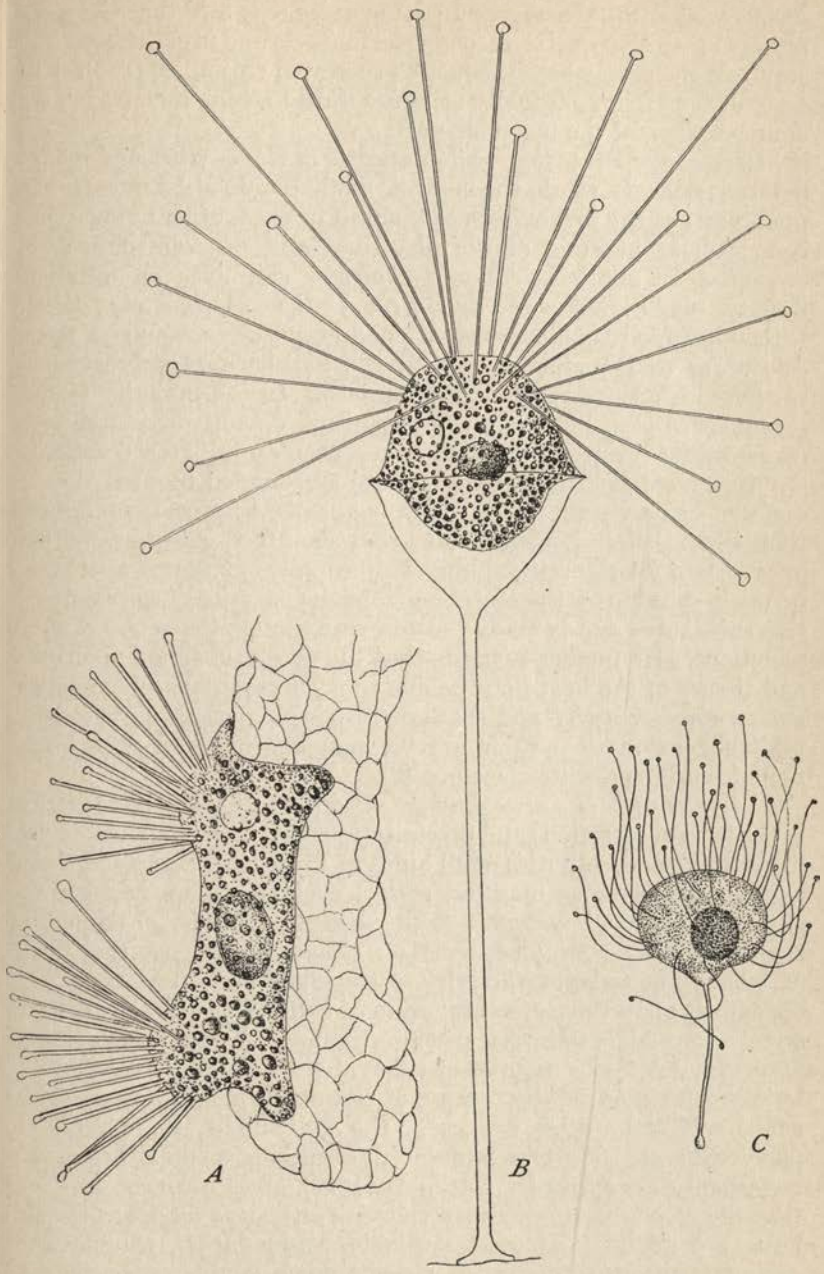

FIG. 91.—Types of Suctoria. *A*, *Trichophrya salparum*, on a gill filament of Salpa; *B*, *Acineta* sp.; *C*, *Podophrya* sp. (Original.)

visible within the tentacle. In some cases it is said that the endoplasm of the captor flows through the tentacle and into the body substance of the prey where the latter is digested (Maupas, 1883). The body of the victim gradually collapses until nothing remains but the denser walls and the insoluble parts.

Many of the Protozoa, while parasitic in the cavities and cells of different animals, retain the holozoic method of food-getting, feeding upon parts of the protoplasm of the host or upon other living organisms such as bacteria of the digestive tract, or solid detritus of one kind or another. Thus *Endamœba coli* lives on intestinal bacteria, while *Endamœba dysenteriæ, Craigia hominis*, etc., engulf, with other food substances, red blood corpuscles and digest them. According to Haughwout (1919), the flagellate *Pentatrichomonas* sp. likewise ingests red blood corpuscles. In the majority of protozoan parasites, however, the organisms do not digest the food necessary for the growth of their own protoplasm. They practically live in a huge gastric vacuole and are surrounded by food already digested or partly digested, which is absorbed by osmosis through their body walls. Doflein thinks that such food substances, if not appropriate for the up-building of protoplasm of the parasite, may be made suitable by the secretion from the parasite of special digestive substances and is ready for absorption after the action of such secretions. He further suggests that the cytolytic action upon cells and tissues of the host may be due to such secretions (for example *Endamœba dysenteriæ*) and that other toxins of pathogenic Protozoa, probably enzymatic in their activity, may be similar digestive secretions from the parasites (see p. 190).

Secretions and Digestive Fluids.—Products of metabolic activity in the form of secretions and precipitations play most important roles in structure and activities of all kinds of Protozoa. Skeletons, shells and tests, gelatinous mantles, stalks, cyst and spore membranes, and the like are all evidences of the secretory activity of the protozoan protoplasm (see Chapter III). There is evidence that these activities, like secretory activity of the gland cells in Metazoa, are dependent upon the general function of irritability and that specific secretory response follows a specific stimulus. Thus Breslau (1921) finds that gelatinous mantles or tubes about *Colpidium colpoda* may be called forth at will by the use of certain chemicals (iodine, fatty acids). If fatty acids are used, the individuals, as in artificial parthenogenesis, must be replaced in a suitable medium before the membranes are formed. Enriques (1919) gives evidence to show that the secretion of stalk material in *Anthophysa vegetans* depends upon the quantity of food available. Stimulation, through the agency of foreign proteins, is without much doubt responsible for the secretion of digestive fluids and ferments in holozoic nutrition, and considerable advance has been made in our knowledge of intra-

cellular digestion. This advance has been due mainly to the application of the method first devised by Gleichen (1778) of introducing into the body with food substances, inorganic, usually colored particles, which clearly outline the limits of the digestive cavities. These cavities, early termed gastric vacuoles, were recognized as digesting centers of the organisms, and Gleichen's method, employed by Ehrenberg (1833–1838) led to his elaborate and at first widely accepted, but erroneous, conception of the Polygastrica. Modern applications of this method consist in the introduction with the food of delicate chemical substances, or indicators, which change in color according to the acid or alkaline nature of the fluids in which they lie. The observations of le Dantec (1890), Fabre-Domergue (1888), Metschnikoff (1889), Greenwood (1887–1894), Nirenstein (1905), Khainsky (1910), and Metalnikoff (1903, 1912), together with the study of extractives by Mesnil (1903), Mouton (1902), Metschnikoff (1893), Krukenberg (1886), Hartog and Dixon (1893), etc., have given a fairly comprehensive idea of the processes of intracellular protein digestion in Protozoa. Another group of observers including Meissner, Greenwood and Saunders, Stolç (1900), Wortmann (1884), Celakowski (1892), Nirenstein, etc., have shown the digestive possibilities in relation to carbohydrates and fats.

The majority of Protozoa which ingest "solid" food take in at the same time more or less water, which forms the gastric vacuole. Thus in trichostomatous ciliates a vacuole is formed at the base of the cytopharynx which varies in size according to the abundance of food particles present. In *Paramecium caudatum* the vacuole, when formed, becomes spindle-shape as though pulled away from the gullet by endoplasmic force, but it soon becomes spherical as it moves about in the fluid endoplasm (Nirenstein, 1905). With the ingestion of larger food bodies such as infusoria, flagellates of larger size, diatoms, rotifers, etc., comparatively little water accompanies the prey. *Paramecium caudatum* when eaten by *Didinium nasutum*, for example, lies in close contact with the protoplasm of its captor and no water at all can be made out (Fig. 89). In such cases the ingested organism is paralyzed and therefore motionless when swallowed, but it very often happens that resistant food bodies continue to struggle after they have been taken into the protoplasm; rotifers, for example, are usually not motionless when engulfed by *Amœba proteus*. In such cases a considerable volume of water gives the prey ample room to move without danger to the make-up of the captor. In other cases in which water does not appear to be taken in with the food, the latter becomes surrounded by fluids secreted by the protoplasm.

With many types of Protozoa the process of digestion begins before the living prey is taken into the protoplasm of the captor.

This is manifested in most cases by the paralysis of the victim when it comes in contact with pseudopodia of many rhizopods and Heliozoa, Ehrenberg (1833) for *Actinophrys sol;* F. E. Schultze (1875–1876) for *Allogromia* and *Polystomella;* Winter (1907) for *Peneroplis*, etc., with tentacles of *Actinobolus radians* Moody (1912) Calkins (1901), or of Suctoria, or with the proboscis of *Didinium, nasutum,* Thon (1905), Mast (1923), Calkins (1915). In some cases, at least, it is not improbable that this paralyzing killing substance is analogous to, if not the same as, the digestive fluids which kill bacteria and other prey after they are taken into the body protoplasm. Thus bacteria become motionless in about thirty seconds after the gastric vacuole is detached from the cytopharynx of *Paramecium caudatum* (Metalnikoff, 1903 and 1912). The color changes of chemical indicators, for example, alizarin sulphate, show that the killing agent is acid in nature; this was early detected by Greenwood and Saunders (1894), who interpreted it as a mineral acid without further specification. Later observers have confirmed this suggestion, Nirenstein, Metalnikoff and others showing that digestion in the vacuole is a process which is divisible into two periods, in one of which the reaction of the vacuole contents is acid, while in the other it is alkaline. The acid reaction lasts for about fifteen minutes, according to Nirenstein and Metalnikoff, in the gastric vacuoles of Paramecium, but Khainsky concluded that the acid reaction is maintained during the entire period of digestion, becoming alkaline only after the dissolution of the protein substances is at an end. In other cases, however, no acid reaction at all can be demonstrated. Thus, Metalnikoff, also in the case of *Paramecium*, found that some vacuoles never give an acid reaction; others much more rarely show an acid reaction throughout, while still others in the same organism are first acid and then alkaline. Minchin (1912) suggests, in connection with this diverse history of vacuoles in the same species, that different food substances incite different responses on the part of the protoplasm much as different antibodies are formed from cells of the Metazoa in response to toxins from different types of pathogenic parasites. No acid has been demonstrated in gastric vacuoles of *Actinosphœrium eichhornii* or in *Amœba proteus* (Greenwood), nor could Metschnikoff find it in *Noctiluca miliaris* or *Euplotes*. This may be correlated with the fact that in the rhizopods and Heliozoa at least the prey is killed upon contact with the pseudopodia, or body protoplasm, the killing agents in such cases perhaps corresponding with the acid secretion of ciliates during the first stages of digestion.

From the number of different ferments which have been isolated from different types of Protozoa, it is quite probable that digestion does not take the same course in all types. Pepsin-like ferments, which dissolve albumins in an acid medium, were isolated by

Krukenberg (1886) from the Mycetozoön *Æthalium septicum*, and by Hartog and Dixon (1893) from the amœba *Pelomyxa palustris*, while Metschnikoff (1889) showed that the food vacuoles in the plasmodia of *Æthalium* have an acid reaction favorable to the activity of such ferments. Trypsin-like ferments have likewise been isolated by Mouton (1902), from soil amœba cultivated in large numbers on agar; also diastatic ferments were easily obtained from *Balantidium coli* by Glaessner (1908), and from *Pelomyxa palustris* by Hartog and Dixon (1893).

The typical course of a gastric vacuole through the endoplasm of ciliates has been carefully worked out by Greenwood and by

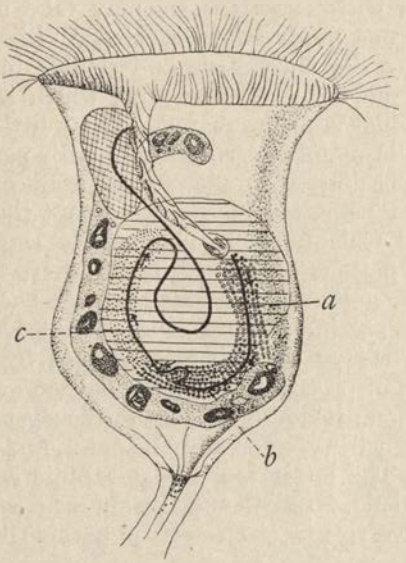

FIG. 92.—*Carchesium polypinum ?* History of food vacuole; (*a*) stage of storage and little change; (*b*) stage of acid reaction; (*c*) neutral reaction. (After Greenwood.)

Nirenstein for *Carchesium* and *Paramecium caudatum* (Fig. 92). Prowazek (1897) staining with neutral red found a collection of red granules about the gastric vacuole; similar granules were observed by him and by Nirenstein (1905) to pass into the gastric vacuole and to mix with the food substances from which circumstance they were regarded by both observers as the bearers of ferments (trypsin-like according to Nirenstein). The so-called Excretperlen (excretory granules) first described by Prowazek (1897) and interpreted by him, by Nirenstein and by Doflein (1916) as furnishing evidence of excretion through the general cell membrane, may be with equal justification interpreted as secretory

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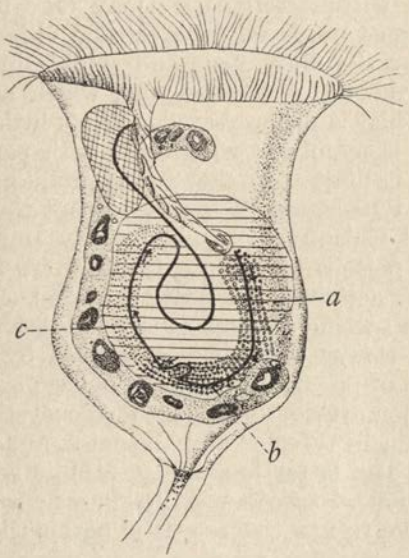

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granules. If the neutral red staining granules about the gastric vacuoles are bearers of ferments as maintained by Prowazek, they certainly are secretory in nature. There is some uncertainty, however, as to the identity of these with the so-called excretory granules. The more recent experiments of Slonimski and Zweibaum (1922) show that there are two types of these granules which they call A and B, and that the peripheral granules (B) which exude from the membrane vary in number and size according to external conditions of temperature and internal conditions of vitality, being rare or absent prior to conjugation. The nature of these varying granules and their function in metabolism are unsolved problems at the present time.

In connection with secretions we may take into consideration the various poisons produced by Protozoa either in the form of toxins exuded by the individuals and soluble in the surrounding medium, or in the form of endotoxins which are liberated only when the individual is disintegrated. What little is known about these secretions is mainly in connection with parasitic forms and here knowledge is limited to the effects produced upon the host. In general it may be stated that, if we except the toxins produced by the so-called Chlamydozoa (particularly smallpox and rabies organisms), the poisons of protozoan origin are much slower and indefinite in their action on the host than are bacterial toxins, and the course of the specific diseases caused by pathogenic protozoa is relatively much slower than diseases caused by bacteria. Relatively few toxins of protozoan origin have been extracted and used in experimentation. One such, called sarcocystin, was obtained from sarcosporidia by Pfeiffer and Gasparck and by Laveran and Mesnil (1899). The latter found that rabbits are soon killed by the blood injection of sarcocystin in glycerin solution, also that crushed cysts give rise to characteristic pathological effects in the muscles, whereas no such reaction accompanies the presence of uninjured cysts.

Filtered blood of malaria victims, if taken at the height of paroxysm and injected into a malaria-free individual, produces in the individual a characteristic malarial paroxysm according to Rosenau and his co-workers, and analogous "paroxysm toxins" have been detected in connection with other blood parasites. Such experiments indicate that toxins from malaria organisms produce rather intensive effects of a generalized character.

Toxins from organisms of amœbic dysentery are more regional in their action, causing local ulceration and abscess formation indicating a cytolytic process possibly due to secretions of digestive fluids. There is still some uncertainty, however, in regard to this matter, and the possibility of participation by bacteria in the reactions is not excluded.

Notwithstanding the serious diseases in man and mammals generally due to trypanosomes, there is very little positive evidence that secretions are responsible for the effects produced. Experiments with extractives from *Trypanosoma brucei* by Kanthak, Durham and Blanford, and by Laveran and Mesnil, gave no indication of toxic effects. On the other hand, Novy and MacNeal, injecting dead *Trypanosoma brucei* in guinea-pigs obtained definite fever symptoms, loss of weight and local ulcerations which, however, they did not trace to the effects of a specific toxin.

Somewhat more positive evidence is accumulating in regard to the possibility of endoenzymes locked up in the trypanosome protoplasm and liberated on disintegration. Thus a number of observers, amongst whom may be enumerated MacNeal, Plimmer, Leber, Martin and others, have interpreted the rise in temperature of organisms with trypanosomiasis as due to the presence of endotoxins, freed in the blood upon death and disintegration of trypanosomes resulting from treatment with medicaments. Also Uhlenhuth, Woithe, Hübener and others have concluded that endotoxins fatal to rats are liberated if blood containing *Trypanosoma equiperdum* is first dried, then dissolved again and injected into rats. Schilling, Braun, Teichmann, on the other hand, got no reaction upon injecting dead pathogenic trypanosomes into the peritoneum or subcutaneously.

In all of these cases, with the exception of sarcocystin, the evidence in favor of the secretion of exotoxins or the presence of endotoxins, is purely circumstantial and verification by chemical and biological methods with exclusion of other possible contributing factors has not yet appeared.

Other indirect evidence of the presence of toxins is furnished by the immunity reactions of different hosts in which the presence of antibodies may safely be inferred. In some cases, *e. g.*, coast fever, many babesiases and various experimental trypanosome infections, the first onset may prove fatal. More frequently, however, protozoan diseases are not fatal at the onset; this is the case with most *Trypanosoma*, *Leishmania* and malaria infections in man and experimental animals. In rare instances after the onset all parasites in the blood are killed, but in the majority of protozoön diseases many of the parasites escape the reactions of the host and continue to live, either in the blood or in some organ where they are partially protected. These are responsible for the relapses and recurrences characteristic of many types of protozoön disease.

As in bacterial diseases, so here the reactions of the host may be against the parasite (bactericidal), against digestive ferments or against poisonous secretions or toxins. In but few cases, however, are the actual substances and the specificity involved in such reactions, known or recognized. In several instances, as Laveran and

Mesnil have shown, human blood serum contains some substance which is fatal to many species of *Trypanosoma* pathogenic in other mammals, but harmless to *Trypanosoma gambiense*, the cause of sleeping sickness. Such protective substances characteristic of natural immunity are developed in the host as a result of infection (acquired immunity) and are fatal to the specific organism causing their formation, or to the toxins produced by the organism. Several such antibodies are demonstrable in the blood serum of the host, after protozoön infection and disease. As shown by experiments of Rabinowitsch and Kempner with *Trypanosoma brucei*, Klein and Möllers, with *Trypanosoma brucei*, Nocard and Theiler with *Babesia*, etc. Further evidence is afforded by the formation of agglutinins called out by the presence of Protozoa. Here the results of Roessle with free-living forms of *Paramecium* and *Glaucoma* are not convincing because of the impossibility of getting these organisms free from bacteria. With parasitic forms, however, the evidence of the presence of specific agglutinins called forth by infecting parasites is fairly strong. The agglomerations of trypanosomes described by Schaudinn, by Laveran and Mesnil, and others for trypanosomes, are examples of this indirect effect of protozoön secretions.

Like the hosts with their immunity reactions, so, too, the protozoan parasites may develop a resistance to the immunity reactions in the form of a counter-immunity. Thus atoxyl-fast, poison-fast, etc., races of Trypanosoma appeared in Ehrlich's experiments, and many observers with free-living protozoa have shown the acquisition of tolerance towards poisons of different kinds, *e. g.*, bichloride of mercury, arsenic, alcohol, etc.

2. *Digestion of Carbohydrates* and *Fats.*—Specific ferments for the transformation of starch into soluble sugar have not been isolated; nevertheless, the evidence that such action takes place is convincing. Curiously enough, this evidence does not apply to the Infusoria where very little digestion, beyond a slight corroding of starch grains, occurs. In rhizopods, however, especially in the amœboid *Pelomyxa* and in species of *Amœba*, starch grains are entirely dissolved, according to the observations of Stolç (1900) who found that the characteristic refringent granules of *Pelomyxa palustris* have a very definite relation to carbohydrate nutrition. These granules (Glanzkörper) are filled with glycogen, the volume of which increases up to fourfold when the animals are fed with starch, and decreases to entire disappearance when they are starved. Even cellulose is said by Stolç to be digested by this organism and Schaudinn made the same observation on the Foraminiferon *Calcituba polymorpha*. In Foraminifera generally, according to Jensen, and in myxomycetes, according to Wortmann, Lister and Celakowsky, starch may be similarly digested. The flagellates, apart from chlorophyll-bearing forms, apparently have in some cases, at

least, the same power of dissolving starch. Thus, *Protomonas amyli* and *Phyllomitus augustatus* eat practically nothing but starch, a fact indicating the action of appropriate digestion ferments. The Hypermastigidæ which are abundant in white ants (termites) are unusual in their ability to digest cellulose. It has been shown that these flagellates live as symbionts with their termite hosts digesting the wood eaten by them by the aid of glycogen. The termites die if deprived by heating of their protozoan symbionts; the protozoa die if the wood diet of the termites is stopped (Cleveland, 1923).

In no protozoön has the actual digestion of fat been observed. Under experimental conditions, ingested fats are carried along unchanged in the protoplasm. We cannot state arbitrarily, however, that fats are not emulsified and used as food. On the contrary, it is difficult to account for the presence of oils and fat bodies in varying quantities in all groups of Protozoa under any other assumption, despite the negative results of Stamiewicz (1910) and of Nirenstein (1909).

3. *Defecation.*—Undigested and indigestible remains are disposed of by discharge into the surrounding medium, well-developed and permanent anal pores occurring in some forms (see supra). Many of the products of assimilation are similarly disposed of by defecation.

(*b*) *Saprozoic Nutrition.*—In holozoic nutrition the food substances are in the form of complex proteins, making up the bodies of the various organisms ingested. In saprozoic and saprophytic nutrition the food substances are less complex chemically, consisting of materials dissolved out of the disintegrating bodies of animals and plants. These are taken in, not through the agency of specialized oral motile organs, nor through a definite mouth, but are absorbed through the body wall in most cases at a special receptive area near the base of the flagellum, as in *Chilomonas paramecium* (Fig. 46, p. 91). Many of the smaller types of flagellates obtain their nutriment in this way, extracts or infusions of animal or plant tissues containing various salts and organic compounds forming excellent culture media for such Protozoa. Nothing is known, however, of the chemical make-up of such fluid substances, nor is it known whether they are prepared for absorption by chemical processes due to the activity of the receptive organism; nor is there any evidence to indicate processes of digestion subsequent to their absorption.

Very little advance has been made in the matter of saprozoic and saprophytic nutrition. The general assumption, based upon the thriving cultures in infusions of disintegrating animal and plant matter, has been that dissolved proteins are taken into the protoplasmic bodies of many kinds of Protozoa by absorption through the general cortex or through some specialized region for the purpose.

The biochemistry of the process is practically unknown, and few experiments on strictly saprozoic forms have been made. Khawkine (1885, 1886) was apparently the first to demonstrate that chlorophyll-bearing flagellates (*Euglena*) can live more or less perfectly as saprozoic organisms. This was further elaborated by Zumstein (1900), who showed that *Euglena gracilis* can live in a colorless and in a chlorophyll-bearing condition. It was long since suggested hypothetically, and later verified experimentally, that organic matters are taken into green flagellates from the surrounding medium. Zumstein endeavored to find out whether such organic matters consist of carbon compounds, or nitrogen and ammonia compounds, and found that in a bacteria-free medium, peptone with the addition of some carbohydrate gave the best results. Ternetz (1912), confirming Zumstein's main results, found that the best sources of nitrogen were asparagin, glycocoll, and alanin, while ammonia compounds were generally detrimental.

From such experiments, it appears probable that saprozoic forms of Protozoa get their main nourishment from amino-acids derived from disintegration of animal and plant matter through the agency of bacteria, and from carbohydrates in solution. The necessary mineral matters are obtained from the surrounding alkaline medium.

In this connection, it is important to consider the possible interaction of excretion products of different Protozoa upon themselves and upon each other, as well as the effects of products of bacterial action. It has long been known that isolation cultures are frequently threatened by the growth of detrimental bacteria. On *a priori* grounds it is not improbable that excretion products of Protozoa themselves may have such an effect. Woodruff (1912, 1913) has studied this problem in connection with *Paramecium aurelia* and the hypotrichous ciliates, *Stylonychia pustulata* and *Pleurotricha lanceolata*, and found that *Paramecium* when placed in filtered medium, which had contained enormous numbers of *Paramecium* in pure culture, were manifestly weakened in vitality. Similarly the hypotrichs when placed in filtered medium which had swarmed with hypotrichs, showed a weakened vitality. When, however, *Paramecium* was placed in filtered hypotrich culture medium, the result was an increased vitality. Woodruff concluded that excretion products from *Paramecium* are detrimental to *Paramecium*, and hypotrich products to hypotrichs, while the latter products have a somewhat stimulating effect on *Paramecium*. This may be, as Woodruff suggests, of some importance in determining the sequence of protozoön forms in a limited environment such as hay infusion.

Parasitic nutrition is not a specific form of nutrition and refers to the effect upon a host, rather than to any physiological activity of the parasite itself. Nutrition of parasites, indeed, may follow

any type of nutrition of free-living forms with the exception of autotrophic nutrition, and even this seems possible with intestinal parasites of the tadpole (Hegner, 1923; Wenrick, 1924). Holozoic nutrition, or the engulfing of solid particles of protein substance, is found in *Endamœba dysenteriæ*, *Craigia hominis*, etc., which ingest bits of tissue and red blood corpuscles. The equivalent of saprozoic nutrition, called osmotic nutrition by Doflein, is a more common form. It is quite possible, although not proved, that some parasites such as the pathogenic amœbæ, secrete proteoclastic ferments which digest tissue elements outside of the amœba protoplasm and then absorb the digested product by osmosis. Such a process might account for the characteristic lesions in the liver or intestine during amœbic dysentery. The majority of protozoan parasites, however, apparently live upon the products of digestion as prepared by the host, the digestive tract being in effect one huge gastric vacuole. Many intracellular parasites intercept similar digested food material destined for tissue cells at the end stage of its journey (Coccidia, Hæmosporidia, hæmoflagellates, intracellular flagellates, Myxosporidia, etc.). Others live upon products of tissue metabolism which are absorbed by osmosis, as in lumen dwelling forms, or on products of cell activity as in hemoglobin absorption by malaria organisms, *Babesias*, etc. In such cases, it is unknown whether the parasite selects particular substances from its environment, or prepares its food by the secretion of digestive fluids.

Specific structural adaptations, useful in such methods of food-getting, are characteristic. Haustoria-like processes, derived from the epimerites of gregarines, in some cases extend deeply in the tissue cell (*Stylorhyncus longicollis*, *Echinomera hispida*, *Pyxinia mœbiuszi*, etc., Fig. 93). The coccidian *Caryotropha mesnili*, according to Siedlecki, shows a significant relation between the nucleus of the host cell and that of the parasite. This organism is a parasite in the spermatozoa of the annelid *Polymnia nebulosa* where the sperm cells are aggregated in bundles in the characteristic annelid fashion, usually about a feeding mass or blastophore. The parasite gets into such a cell as an agamete or sporozoite, one only of the bundle, as a rule, being infected, and as it grows the nucleus of the cell is displaced to one side and the cell loses its characteristic structure, becoming hypertrophied and distorted (Fig. 93, 2). Not only the infected cell but all the other cells of the spermatogonia bundle are affected, and none of them continues the normal development, but they become arranged like epithelial cells about the hypertrophied infected cell.

The specific effect of the young *Caryotropha* on the infected cell consists not only of the enlargement of that cell, but of a definite feeding mechanism by which the parasite is supplied with food. That the nucleus is a center of constructive metabolic changes is

well assured at the present day, and the conditions in these parasites suggests the peculiar relation which Shibata (1902) has described in the intracellular mycorhiza, where a mycelium thread is grown straight toward the nourishing cell nucleus of the host, causing marked hypertrophy on the part of the cell. In *Caryotropha,* the nucleus of the host cell is pushed to one side and the parasite assumes such a form that the nucleus lies in a small bay (Fig. 93, 2*n*). In the cytoplasm of the cell an intracellular canal is then formed which runs from the host nucleus to the nucleus of the parasite, and Siedlecki holds that the food of the parasite is all elaborated by the nucleus of the host cell, while the other spermatogonia

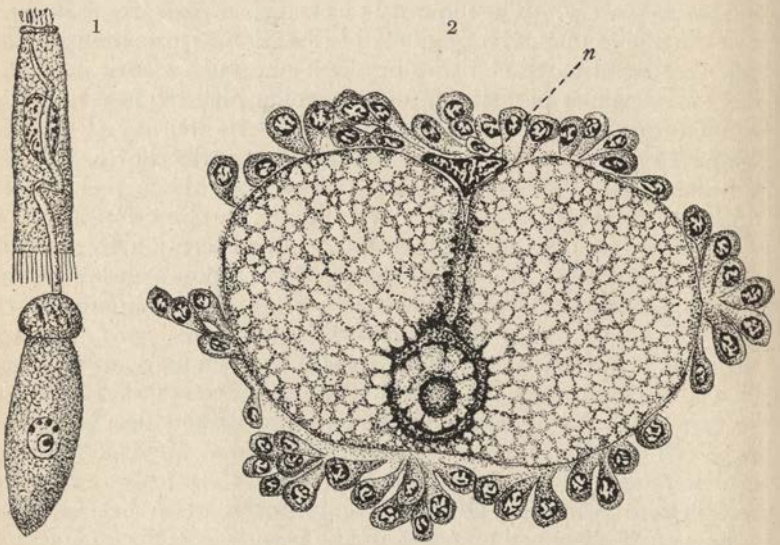

FIG. 93.—Food-getting adaptations of Sporozoa. 1, *Pyxinia mobiuszi* with epimerite deeply insunk in the epithelial host cell (after Léger and Duboscq); 2, *Caryotropha mesnili* with an intracellular canal from the nucleus of the host cell (*n*). (After Siedlecki.)

form a protective epithelial sheath around it. When the parasite is full grown the cell is destroyed and the bundle degenerates.

It is difficult to draw the line between symbionts, commensals and parasites. Symbionts are organisms living with a host in such a relation that both are benefited; commensals are organisms which live with a host without benefit or injury to the latter but to their own advantage, and parasites are organisms which, to their own benefit, cause injury in one form or other to the host. Symbiosis is well illustrated by the harmonious life of some chlorophyll-bearing forms, Zoöchlorella, Zoöxanthella, etc., and Protozoa in which the former live (*Paramecium bursaria,* "yellow cells," *Stentor viridis,*

Amœba viridis, *Vorticella viridis* and Radiolaria etc.), and it is conceivable that some gut-dwelling forms may perform a useful activity for a host by disposing of pernicious bacteria, or by preparing food substances for use by the host as do Hypermastigidæ in termites (Cleveland). Commensals, such as *Endamœba coli*, *Endamœba nana*, *Trichomonas* species and other intestinal forms may, on occasions, turn into parasites, as is the case with *Trichomonas* (*Tritrichomonas*, Kofoid), *Giardia* (*Lamblia*), etc. Musgrave and Clegg, indeed, are skeptical of any amœba that may get into the intestine, taking the view that any free-living form capable of adapting itself to conditions of a digestive tract, may adapt itself to a mode of life injurious to the host.

Parasites upon reaching a site where the environmental conditions of food, etc., are suitable, begin to multiply and to accumulate, thus giving the appearance of selecting a given organ or tissue. In this way, the organisms of smallpox (*Cytoryctes variolæ*) are characteristic parasites of the chorium; those of rabies (*Neuroryctes hydrophobiæ*) are nerve tissue parasites, while *Plasmodium*, *Proteosoma*, *Leishmania*, *Trypanosoma*, etc., are typical parasites of the blood and lymph; Coccidia are intracellular in various tissues which are specific for each type of parasite, the particular habitat in all cases, depending on the food conditions and the physiological reactions of the host. Such habitats have led to the designations of parasites as cœlozoic (lumen dwelling), enterozoic (gut dwelling), histozoic (tissue dwelling), cytozoic (intracellular), and hematozoic (blood dwelling) forms. Many of them combine two or more of these phases during the life cycle. Thus gregarines are cytozoic in youth, and cœlozoic later in life; some flagellates (*Leishmania; Trypanosoma*), are hematozoic and cytozoic; others are enterozoic and histozoic (*Sarcocystis*), and some are cœlozoic in one host and hematozoic in another (malaria organisms).

(*c*) *Autotrophic Nutrition.*—Heterotrophic nutrition of all animals is possible only where organic foodstuffs are present and such foodstuffs, in the final analysis, are manufactured by chlorophyll-bearing plants. Many Protozoa, particularly flagellates, are provided with this manufacturing outfit which appears in typical green chlorophyll color in Euglenida and Phytomonadida. In many cases (Chrysomonadida, Cryptomonadida), the green color is masked by yellow or brown pigment which is easily dissolved in weak alcohol leaving the green chlorophyll exposed; or the color may be blue-green as in the rhizopod *Paulinella*. Green chlorophyll resembles plant chlorophyll in all respects—but yellow chlorophyll, especially the phycopyrin of the Dinoflagellida, is closely similar to the yellow coloring matter of diatoms (diatomin). In many cases (*Euglena sanguinea*, *Hæmotococcus pluvialis*, and *Chlamydomonas nivalis*) the green is masked by a red hematochrome termed karotin, which is

probably a modified form of chlorophyll, the transition being brought about by scarcity of nitrogen or phosphorus. "Red snow" or "bloody pools" owe their origin to masses of these flagellates colored by karotin, which, according to the observations of Reichenow (1909) disappear from alpine red snows or pools in summer when, through decaying vegetation, the waters are richer in organic compounds.

Little is known accurately of the method by which organic foodstuffs are manufactured by chlorophyll and practically nothing is known about the process in Protozoa. Presumably the activities here are the same as in the higher plants, food manufacture being a result of photosynthesis. The spectrum of chlorophyll shows absorption particularly of the short wave rays of light—notably blue and green regions of the white light spectrum, and in some way not yet understood, the kinetic energy of sunlight, transformed into potential energy of chlorophyll, is utilized in the synthesis of organic compounds. Carbon, hydrogen and oxygen are essential for carbohydrate synthesis, and nitrogen must be added to form protein. Such combinations require energy and undoubtedly the energy obtained from sunlight supplies this need. While CO_2 and H_2O are essential for plant activity, little is known of the exact manner in which they are essential. Also, while $C_6H_{10}O_5$ or starch may be derived on paper by combining 6 molecules of the one and 5 of the other, the exact process is unknown, and the chances are that it is not so simple as appears by the equation. The sensitiveness of chlorophyll, or its extreme lability in light and darkness, its first appearance only in the light, are factors indicating an intimate physiological dependence upon the radiant energy of the sun. It is not altogether satisfactory to assume that chlorophyll uses this energy as one would use a tool, to separate the elements of CO_2 and H_2O, and to unite them again into CH_2O or formaldehyde, and then to use it again in the condensation of CH_2O into $C_6H_{12}O_6$ or sugar; or to use it directly for condensing H_2CO_3 into $C_6H_{12}O_6$ and O_2. The instability of chlorophyll; its disappearance under unfavorable and reappearance under favorable conditions, leave little basis for the assumption that it remains unchanged throughout the reactions which it is responsible for bringing about. The experiments of Jörgensen and Kidd (1916) whereby extracted chlorophyll in sunlight produced no formaldehyde in an atmosphere of pure CO_2, but did produce it in an atmosphere of pure oxygen, indicate that formaldehyde formation and production of carbohydrates in plants may be a result of oxidation and not of synthesis in atmospheric air.

If Wilstätter's formula for chlorophyll is approximately correct we have a protein molcule thus—($MgN_4C_{32}H_{30}O$) ($COOCH_3$) ($COOC_{20}H_{39}$) in which the chromogen radical ($MgN_4C_{32}H_{30}O$) may

be separated by oxidation from the alcohol groups and the latter broken up into free oxygen and formaldehyde or directly into sugar. Some such process apparently occurred in the experiment cited, but no synthesis of sugar or regeneration of the chlorophyll molecule took place. On the contrary, the chromogen material was soon broken down through displacement of the magnesium by hydrogen, due to the action of the increasing quantity of formic acid and the reaction stopped. In the living plant it is conceivable that through energy from sunlight, formaldehyde, if formed, is condensed to sugar, while the nitrogen-holding compound, chromogen, forms again the complex chlorophyll molecule by regeneration through union with CO_2 and H_2O with the aid of energy from sunlight. On this hypothesis, starch or sugar formation is a result of protein metabolism acting with the energy of sunlight, while CO_2 and H_2O are foods or raw materials necessary for upbuilding in chlorophyll regeneration. Protein metabolism in plants and animals would thus be placed on a similar basis, katalytic action breaking down the complex protein molecule, giving rise to a metaplastid starch and a chromogen radical capable of taking on raw materials (food) necessary for its regeneration. On such an hypothesis the essential use of CO_2 and H_2O would be as food for the plant in building up its particular type of protein—*viz.*, chlorophyll, and until that chlorophyll is formed no starch or sugar is produced.

(*d*) *Heterotrophic Nutrition.*—The ability of certain organisms to live on manufactured foods in the light and to live equally well on proteins manufactured by other living organisms, has been known for many years. Bütschli called attention to it in the case of *Chromulina* (1884) and in some Dinoflagellates; Ternetz (1912) and Zumstein (1900) demonstrated experimentally that *Euglena gracilis* can live almost equally well in the light or in the absence of light, and more recently Pascher has shown that practically all of the Chrysomonadida possess this power, while the assertion is made and practically substantiated by experiment, that "all colored flagellates incline to saprophytism and combine in Nature almost regularly both types (holophytic and saprophytic) of nutrition" (1914, pt. I, p. 11). Indeed, the experiments of Zumstein and of Ternetz show that with exclusively organic nourishment *Euglena* races appear in which the chromatophore apparatus is temporarily gone, and Ternetz, at least, succeeded in cultivating races of *Euglena gracilis* in which the chromatophores were said to be permanently lost.

It is quite probable that saprophytic flagellates have been derived through forms with the double or combined modes of nutrition from the strictly autotrophic types. Pascher states, in this connection: "Flagellated forms are present which possess distinct but reduced chromatophores incapable of extensive functions;

others possess no chromatophores at all, but still retain the pyrenoids characteristic of their colored allied forms (*Tetrablepharis*); others retain the stigmata characteristic of chlorophyll-bearing types, but possess no chromatophores, and still others possess neither stigmata, pyrenoids nor chromatophores, but contain assimilation products which are characteristic of the most nearly related colored forms (*Chilomonas* and *Cryptomonas*, *Polytoma* and *Chlamydomonas*)." Loc. cit. p. 11.

In addition to combined autotrophic and saprophytic modes of nutrition, some types of flagellates, especially amongst the Chrysomonadida combine holophytic nutrition with holozoic. Here, in the simplest cases, the intake of solid particles is effected by pseudopodia, either lobose in type or branched (rhizopodia). These may

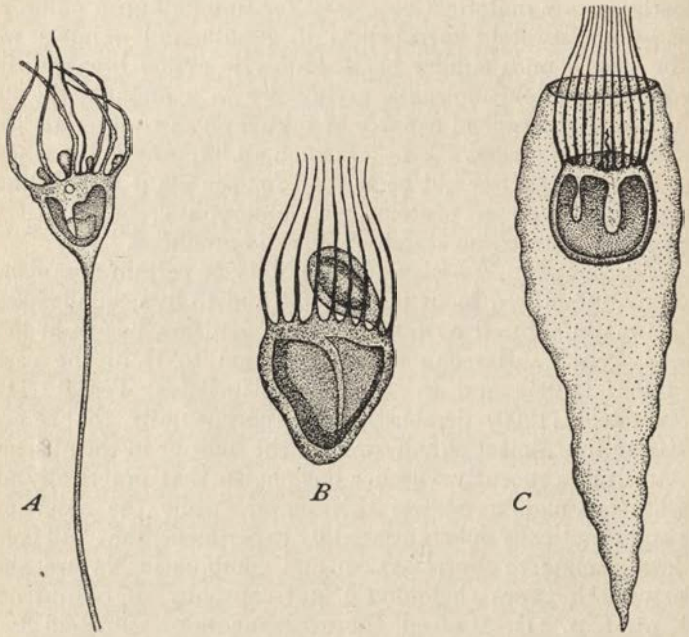

FIG. 94.—*Cyrtophora pedicellata* and *Palatinella cyrtophora*; flagellates with tentacles and exogenous buds. (After Pascher.)

arise from any part of the cell or may be, with the gastric vacuole, confined to the anterior end as in *Dinobryon* (Fig. 126, p. 259). More complex and more differentiated pseudopodia are found amongst the Cyrtophoridæ of the Chrysomonadida. In *Cyrtophora pedicellata* the cell body, with its single cup-shaped yellow chromatophore, is in the form of an inverted pyramid attached by stalk at the apex while the broader anterior end bears a single flagellum and

a crown of tentacle-like pseudopodia, which like axopodia bear an axial filament (Pascher). These pseudopodia serve to capture larger food bodies, while bacteria are caught in the plastic, flowing protoplasm surrounding the axial filament. Somewhat similar forms are found in the genera *Palatinella* of Lauterborn and *Pedinella* of Wysotzki (Fig. 94). An interesting case of parasitism on the part of green chlorophyll-bearing flagellates, *Euglenomorpha hegneri*, has been described by Hegner (1923). The flagellates are found in the intestine, and particularly in the rectum of tadpoles of frogs and toads. Their inability to live outside of this habitat indicates a combination of autotrophic and saprozoic nutrition.

The number and variety of these adaptations for heterotrophic nutrition in addition to the autotrophic and apparently primary nutrition, lend considerable support to Pascher's theory that the colorless flagellates and possibly other Protozoa as well have been derived from chlorophyll-bearing forms (see Pascher, 1916), or to Victor Franz's (1919) view that all Protozoa have been derived from many-celled plant types.

2. **Products of Assimilation.**—These usually appear in the form of storage granules of one type or other, and are dependent upon the mode of nutrition and the kind of food used. In holophytic forms the products are by no means always the same, but they appear to be more or less characteristic for the different groups. Thus in Chrysomonadida leucosin granules are the most typical, while fats and oils are widely distributed (see *supra*); in Cryptomonadida paramylum and other starch-like carbohydrates are characteristic while starch grains are present in the higher types. In Euglenida the characteristic products are paramylum, and in Phytomonadida, true starch.

With the majority of forms the products of assimilation vary with the type of food used and are frequently so abundant in the cell as to give a characteristic appearance or color to the animal. Thus the refringent granules of *Pelomyxa palustris* (Stolç) produce a peculiar refringent effect. The brown granules of *Plasmodium* species, characteristic of malaria, are products of hemoglobin assimilation. Similarly the coccidin of Coccidia; peridinin of Dinoflagellida; stentorin of *Stentor cœruleus* and *Folliculina ampulla;* the pink of *Holosticha;* the lavender of *Blepharisma undulans* or the red of *Mesodinium rubrum,* are examples of the great variety of colored cellular substances dependent upon the food that is eaten. In the absence of the specific kinds of food which yield these chromic products the organisms are colorless, and colored or colorless individuals of the same species may appear in the same culture.

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CHAPTER V.

REPRODUCTION.

GENERAL REPRODUCTION; ALL REPRODUCTION CELL DIVISION.

Of all the marvels associated with the Protozoa there is nothing more staggering to the imagination than the fixity of type which their protoplasm manifests. The genotype, subject to minor variations of a fluctuating character in the course of a normal life history, or subjected experimentally to all kinds of unusual environmental conditions, remains fundamentally unchanged. Types modified through amphimixis or through permanent modifications of the environment may lead to divergent types. This conservatism or fixity of type is a function of the organization which has been continuous in the past and will be continuous in the future. The activities which take place in the organization, the sum total of which constitute vitality, are discontinuous, they have been and will continue to be dependent upon the interactions between organization and environment.

The single individual which we study under the microscope has had no such history in the past and no promise for the future; its span of life as an individual is measured by hours or days only. It is the temporary trustee of a small portion of an organization which has been parceled out amongst unknown myriads of similar trustees. Its metabolic activities are the interactions within the organization and as a result of these activities the fluctuating variations characteristic of the genotype follow one after another in the form of inevitable differentiations which may or may not be visibly indicated by structural changes (see Chapter X). Ultimately its possibilities of further vitality as a single individual are exhausted and it undergoes its final manifestation of vitality. The significance of this final act is a function of all genotypes and of all organizations whereby the organization is further parcelled out to two or more trustees. It is reproduction by division, which by reason of its universal occurrence is one of the most characteristic properties of protoplasm.

There is no doubt that division of the cell is a phenomenon of deep-reaching significance; we shall endeavor to show that the organization as parcelled out to the descendants by division is not a mere equal division of the protoplasm of the individual with its load of metaplastids and other modifications of the organization,

but a renewed or purified organization such as the individual received when it was formed. With the processes of division the old differentiations are lost by absorption, the organization is de-differentiated and the protoplasm has a renewed potential of vitality.

In order to understand the relations of division to the chain of metabolic activities we should know more about the conditions under which division occurs, and the "causes" of division. There is very little real evidence for conclusions in this matter but there have been many theories. The latter for the most part are based either upon analogies with physical phenomena or upon hypothetical "spheres of influence" of morphological elements of the cell. They have been developed in the main to interpret phenomena of division in metazoan cells, particularly in egg cells, and fall completely to the ground when applied to division of Protozoa. So it is with the contractility hypothesis of Heidenhain, Drüner and others who see in the spindle fibers and astral rays a contractile system whereby the nucleus and cell are divided in a strictly mechanical manner. The intranuclear spindle and the absence of cytoplasmic rays in the great majority of Protozoa are enough to show that such physical interpretations do not reach to the root of the matter. The "spheres of influence" hypotheses, based upon the kinetic center of the cell and its influence on the cytoplasm, was developed by Boveri in the attempt to associate cell growth and the causes of division. The "energid" theory of Sachs and Strasburger was an analogous effort to trace the causes of cell division to increasing volume of the cell through growth, each nucleus having its sphere of influence in the cytoplasm and dividing when the volume of the cell outgrows the sphere of activity of the nucleus. The *Kernplasmverhältnis* theory of Hertwig was based upon somewhat similar grounds. According to this the volume of the nucleus bears a certain normal relation or ratio to the volume of the cytoplasm in young actively functioning cells, evidence of which in *Frontonia* was given by Popoff (1909) and by Hegner (1920) in the equidistant distribution of nuclei in various species of *Arcella*. With increasing age this ratio is altered to the advantage of the cytoplasm until division of the cell restores the normal ratio. With uninucleate forms such as *Paramecium* or *Frontonia* there is some evidence of change in relative volumes, and careful measurements by Popoff (1909) and other followers of Hertwig are adduced to support the hypothesis. In these forms the volume of the nucleus is proportionally reduced until just prior to division when the nucleus rapidly increases in volume and divides. In *Uroleptus, Uronychia* and similar forms, however, the many nuclei fuse to form one compact and relatively small nucleus prior to division. It would seem that such changes in relative volume of nucleus and cytoplasm are better interpreted as the effects of underlying conditions which cause division rather than as the cause of division themselves.

None of these theories is of much value in analyzing the antecedent phenomena of division. These must be sought in the reactions of different substances constituting protoplasm. Division of the cell itself is a last step in a progressive series of reproductive changes affecting the entire protoplasm, and constituents of which—microsomes, mitochondria, plastids, chromomeres, kinetic elements, etc.—have already divided. It is in the division of these fundamental granules in the make-up of protoplasm that we must look for the underlying causes of cell division. The dependence of the succession of division processes which characterize reproduction upon growth and metabolism is clearly evidenced by simple starvation experiments, division ceasing with cessation of metabolic activities. There is a possibility that environmental conditions play a more direct part in reproduction than is indicated by their relations to metabolism. Thus Robertson (1921) concludes that a catalase (X substance) is secreted by the living cell which directly enhances division. He found that two individuals, or more, of *Enchelys farcimen* in a drop of culture medium would divide from four to sixteen times more rapidly than a single individual in a similar drop, the result being interpreted as due to contiguity of individuals. This, however, is a direct contradiction of Woodruff's (1911) results with *Paramecium* and *Stylonychia*, according to which the division rate is reduced by accumulation of products of metabolism in the medium. Nor is Robertson supported by other observers. Cutler (1924) for example, found for *Colpidium colpoda* that the division rate depends upon the number of bacteria present as food, and that increase in number of individuals in a drop means a decrease in the individual division rate. Greenleaf (1924) similarly found that solitary individuals of *Paramecium caudatum*, *P. aurelia* and *Pleurotricha lanceolata* isolated in 2, 5, 20 and 40 drops of medium, gave a highest division rate in five days in the 40-drop test, the lowest in a 2-drop test. Also in *Uroleptus mobilis*, in a sixty-day test in which 1 individual, 2, 3 and 4 individuals were isolated daily in a single drop of medium the highest division rate was shown by the solitary individual in a drop as shown in the following table:

10 individuals, 1 to a drop, each divided in the sixty days	74.1 times
20 individuals, 2 to a drop, each divided in the sixty days	59.5 "
30 individuals, 3 to a drop, each divided in the sixty days	54.7 "
40 individuals, 4 to a drop, each divided in the sixty days	54.2 "

Environmental conditions which alter the permeability of the cell, thereby enhancing or retarding metabolic activities do, however, have a corresponding effect upon the division rate. Age of individuals, or the protoplasmic organization at different periods of the life cycle likewise has a determining effect on the rate of division, the differences, as shown in the following table, being due to the differences in the reactions of the protoplasm to the same medium under

different conditions of organization. Series 111 and 112, for example, were 279 and 263 generations old at the beginning of the experiment, the single individual isolated daily in a drop of medium divided 60 times in sixty days. Series 120 and 121 were 12 and 10 generations old, and each solitary individual divided 86 and 107 times in the same sixty days.

UROLEPTUS MOBILIS—DIVISION RATE.

Experiment from September 24 to November 10, 1924.

Series.	Age. Generation.	No. in drop.	Divisions per individual.						
			First, ten days.	Second ten days.	Third, ten days.	Fourth, ten days.	Fifth, ten days.	Sixth, ten days.	Total, sixty days.
111	279	1	12	7	10	13	9	9	60
		2	11	7	6	10	5	5	44
		3	9	5	6	7	5	4	36
		4	10	4	3	6	3	5	31
112	263	1	14	14	9	10	7	6	60
		2	11	13	5	8	4	6	47
		3	13	8	4	7	2	4	38
		4	8	11	10	7	2	3	41
114	160	1	11	8	5	9	4	6	43
		2	6	8	3	6	1	6	30
		3	5	4	3	4	2	0	18
		4	8	4	3	2	3	1	21
115	247	1	14	17	9	10	13	10	73
		2	10	13	6	9	10	9	57
		3	14	16	7	8	8	4	57
		4	15	13	7	9	10	7	61
116	189	1	13	14	10	9	7	7	60
		2	9	10	8	10	9	8	54
		3	9	11	5	7	8	7	47
		4	7	7	3	7	6	4	34
117	133	1	16	18	11	10	14	12	81
		2	14	17	7	10	8	9	65
		3	14	17	8	9	10	9	67
		4	13	17	6	8	10	8	62
118	140	1	18	22	12	16	17	14	99
		2	18	14	8	11	13	13	82
		3	15	20	9	12	11	9	76
		4	14	20	7	12	12	12	77
119	110	1	15	19	10	10	10	8	72
		2	15	14	7	7	7	9	59
		3	11	14	7	8	6	6	52
		4	10	14	6	8	7	5	50
120	12	1	18	19	11	13	13	12	86
		2	16	16	6	12	9	10	69
		3	17	15	5	8	13	9	67
		4	16	15	9	9	13	11	73
121	10	1	18	23	13	16	18	19	107
		2	14	24	9	8	15	18	88
		3	15	23	10	11	14	16	89
		4	19	21	10	11	14	17	92

Each substance entering into the composition of living protoplasm must manufacture new substance of its own kind. All such substances, usually in the form of granules, grow to a certain limit of size and each then divides. Evidence for this is apparent only in the more obvious of the protoplasmic elements such as plastids, kinetic elements, chromomeres, etc., the division of which has been mentioned in the preceding pages. Finally the grand aggregate, the cell itself, divides as a last expression of the series of events that have taken place. It is evident that such division of the cell as a whole constitutes only a small part of the phenomena of reproduction and perhaps not the most important part. While most of the elementary granules, apart from those enumerated above, which make up the bulk of protoplasm, cannot be followed from their smallest stages to the stage when they become visible, it is not inconsistent with the idea of continuity from generation to generation to regard even the smallest as retaining its integrity and reproducing itself by division. "For my part I am disposed to accept the probability that many of the these particles, as if they were submicroscopical plastids, may have a persistent identity, perpetuating themselves by growth and multiplication without loss of their specific individual type" (E. B. Wilson, 1923).

While the division of a single granule results in the formation of two probably identical granules of the same substance, the division of aggregates of granules of different substance may or may not result in identical daughter aggregates. The nucleus is such an aggregate which, by ordinary equations division, is probably divided into two identical halves, but in meiotic divisions the products of the nucleus are different, visible evidence of which is shown by the history of the sex chromosomes and by the results in modern genetics. It is entirely possible that differentiations may arise from such inequalities in nuclear division (see Chapter XII).

The cytoplasm of the cell, likewise, is such an aggregate, made up of all the different substances variously distributed, which compose living protoplasm. If all the granules were equally distributed at division to the daughter cells, as are nuclei and many kinetic elements, then the products of cell division might be identical. Morphological evidence that all granules are not thus equally distributed is furnished by all budding and spore-forming types, and by forms like *Dileptus anser* or *Holosticha multinucleata*, where the large chromatin granules, while still in the process of division, are carried bodily to one or the other daughter cell (Fig. 58, p. 116).

Reproduction whereby a type of organism is perpetuated and distributed, is thus preëminently a process of division. In the last analysis cell division is the only kind of reproduction known. Potential individuals are contained in every germ cell, but germ cells, like other cells, are formed by division and it follows that every

female reproduces as many potential offspring as eggs. Development of such eggs, however, is usually dependent upon fertilization which is quite a distinct phenomenon, accessory to reproduction in most animals, but not itself reproduction. In the present chapter only a summary of the more obvious processes of reproduction will be described, leaving the problems associated with fertilization for treatment in a later section (see Chapter XI).

It is division of the grand aggregate of protoplasmic substances, *i. e.*, division of the cell itself, that is usually described as reproduction of the Protozoa. Such reproductions are usually classified as division, budding or gemmation, and sporulation, the inference being that these are different modes of reproduction. In reality, however, they are different types of reproduction by division, and such modifications would be expressed better by the terms equal division, unequal division, and multiple division.

I. EQUAL DIVISION AND EVIDENCE OF REORGANIZATION.

In the ordinary metabolic processes of an active protozoön there is evidence of a cumulative differentiation which indicates a difference in organization between a young cell immediately after division by which it is formed and the same cell when it is mature and ready itself to divide (see Chapters III and X). Child (1916) mainly from experiments with cells of the Metazoa, came to the conclusion that "senescence consists in a decrease in metabolic-rate determined by the change in, and the progressive accumulation of, the relatively stabile components of the protoplasmic substratum during growth, development and differentiation" (p. 333). He further suggested that in every cell division in unicellular animals, with the accompanying processes of reorganization, there is some degree of rejuvenescence, and if such rejuvenescence balances the cumulative differentiation, continued life of the organisms by division alone may go on indefinitely. By proper conditions of the environment it is conceivable that such a balance may be established. On such an hypothesis it is possible to account for the continued vitality of animal flagellates in which fertilization processes are unknown, for the continued life of many of the higher plants, and for the continued life of the tissue cell cultures in the hands of Carrel and others (see Chapter X).

In many Protozoa there is unmistakable evidence of such reorganization processes which will be described in the following pages; in many there is no visible evidence, but in such cases and in the absence of other possibilities of reorganization, it is permissible to assume that reorganization processes which escape the most vigilant watchfulness of the observer, do actually occur. For descriptive purposes, and on grounds of expediency, the division phenomena

are grouped according to the distribution of the three main types of the Protozoa-Mastigophora, Sarcodina and Infusoria.

A. Division and Reorganization in Mastigophora.—With very few exceptions division in flagellates is longitudinal, beginning as a rule at the anterior or flagellar end, the cleavage plane passing down through the middle of the body. As the halves separate the two daughter cells usually come to lie in one plane so that final division

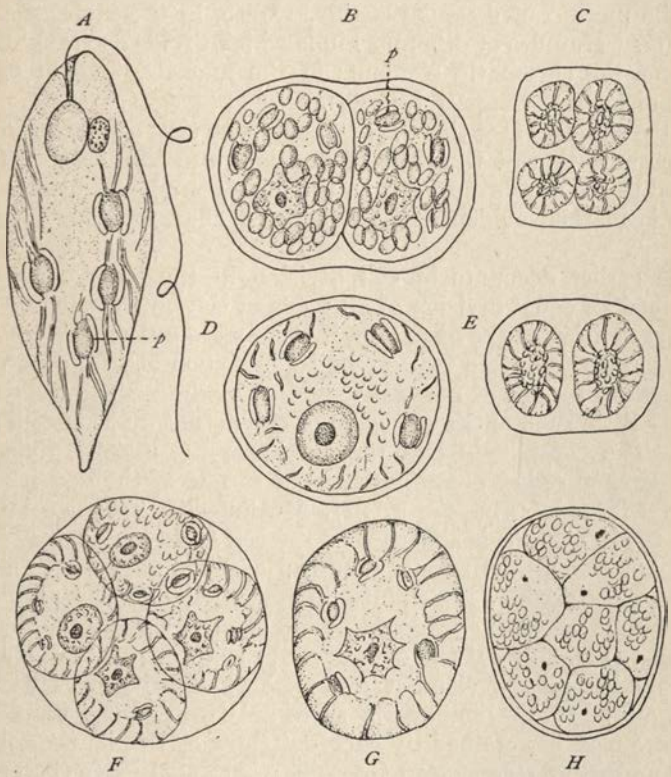

FIG. 95.—*Euglena sociabilis* Dang. Vegetative individual (*A*) and simple and multiple division within cyst. (After Dangeard.)

appears to be transverse. In *Oxyrrhis marina* division is actually transverse (Fig. 43, p. 88), and transverse or oblique in the Dinoflagellida generally. In the majority of forms the individuals divide while freely motile, but this is by no means universal, variations in this respect occurring in the same family and even in the same genus (see Dangeard, 1901). Thus in Euglenidæ division in the motile state occurs in some species of *Euglena* (*E. viridis, E. geniculata, E. flava,* etc.), in *Peranema, Entosiphon, Menoidium, Astasia,*

and others, or in a quiescent but not encysted condition in other species of *Euglena* (*E. spirogyra, Phacus pleuronectes,* etc.); or in encysted stages in which division may be binary (*Euglena deses, Phacus ovum,* etc.) or multiple as in species which give rise to Palmella forms (*E. sociabilis,* etc., Fig. 95).

As there are few details in the structure of a simple flagellate on which to focus attention, descriptions of division processes are practically limited to the history of the nucleus, kinetic elements and the more conspicuous plastids. Here, in the main, are fairly prominent granules of different kinds which divide as granules, and, save for the chromatin elements of the nucleus, without obvious mechanisms (see Chapter I, p. 43).

In the simpler cases there is little evidence that can be interpreted as reorganization at the time of division, and the little we find is limited to the motile organs. In the more complex forms, however, there is marked evidence of deep-seated changes going on in the cell.

The earlier accounts of cell division in the simpler flagellates described an equal division of all parts of the body including longitudinal division of the flagellum, if there were but one, or equal distribution if there were two. One by one such accounts have been checked up by use of modern technical methods until today there is very little substantial evidence of the actual division of a flagellum. The basal body and the blepharoplast usually divide, but the flagellum either passes unchanged to one of the daughter cells as in *Crithidia* (Fig. 48, p. 97), McCulloch) *Trypanosoma,* etc. (Fig. 97, p. 212), or is absorbed in the cell as in *Scytomonas subtilis* (Fig. 96, Dobell). In some doubtful cases it may be thrown off. If the old flagellum is retained in uniflagellate forms the second flagellum develops by outgrowth from the basal body or the blepharoplast (Fig. 96). If the old flagellum is absorbed, both halves of the divided kinetic element give rise to flagella by outgrowths (Fig. 59, p. 117). Similarly if there are two or more flagella, one or more may be retained by each daughter cell while the other, or full number are regenerated (Fig. 98, p. 212). In some cases, as in *Herpetomonas musca-domesticæ,* the regeneration of a second flagellum occurs before division of the cell is evident, a circumstance which evidently led Prowazek (1905) to conclude that this organism is normally bi-flagellated (Fig. 138, p. 289).

Reorganization is indicated to some extent by these cases in which the old flagellum is absorbed. It is also evident in those forms of Chrysoflagellida, Cryptoflagellida and Euglenida which reproduce in the palmella or quiescent phases after the exudation of a gelatinous matrix (see Chapter I), and after loss of the characteristic swimming organs. It is still better indicated by a number of flagellates in which the cytoplasmic kinetic elements, as well as the

flagella, are all absorbed and replaced by new combinations in each of the daughter cells. Thus in *Spongomonas splendida*, according to Hartmann and Chagas (1910) the old blepharoplasts and the two flagella are absorbed and new ones are derived from centrioles of the nuclear division figure (Fig. 59, p. 117). The same phenomenon is described for *Polytoma uvella* (Dangeard, Entz), for *Chlamydomonas* (Dill, see Oltmanns) and *Parapolytoma saturna* (Jameson). The phenomenon cannot be regarded as typical of the simple flagellates, for in the great majority the kinetic elements are self-per-

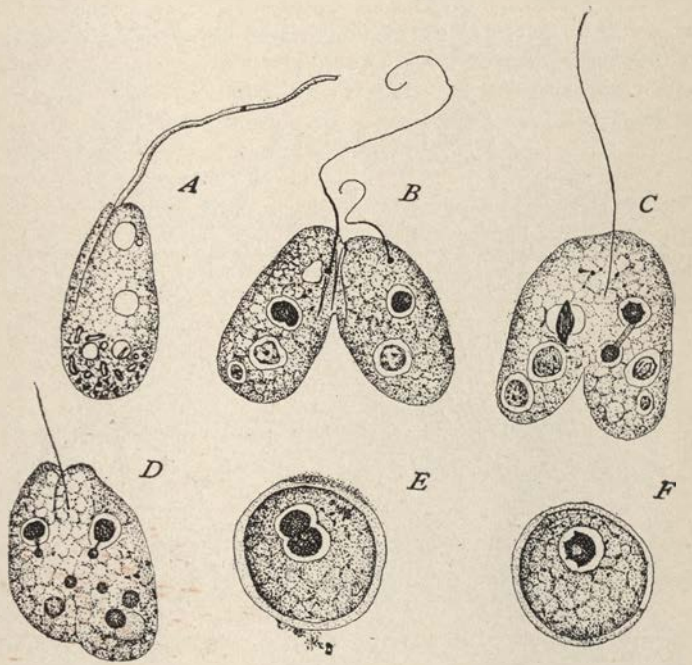

FIG. 96.—*Scytomonas subtilis*, hologamic copulation. *A*, normal adult individual *B* to *F*, successive stages in fusion, loss of flagella, and encystment. (After Dobell.)

petuating, even the axostyles according to Kofoid and Swezy (1915) dividing in *Trichomonas* (Fig. 72, p. 139). This, however, is not supported by Wenrich (1921).

An extreme case of reorganization is apparent in the two species of *Lophomonas* (*L. blattæ* and *L. striata*) first described by Janicki (1915). Here the parental calyx, basal bodies, blepharoplasts and rhizoplasts all degenerate during division (Fig. 98). At division a cytoplasmic centriole first divides with a connecting fibril which is retained throughout as a paradesmose. The nucleus emerges from

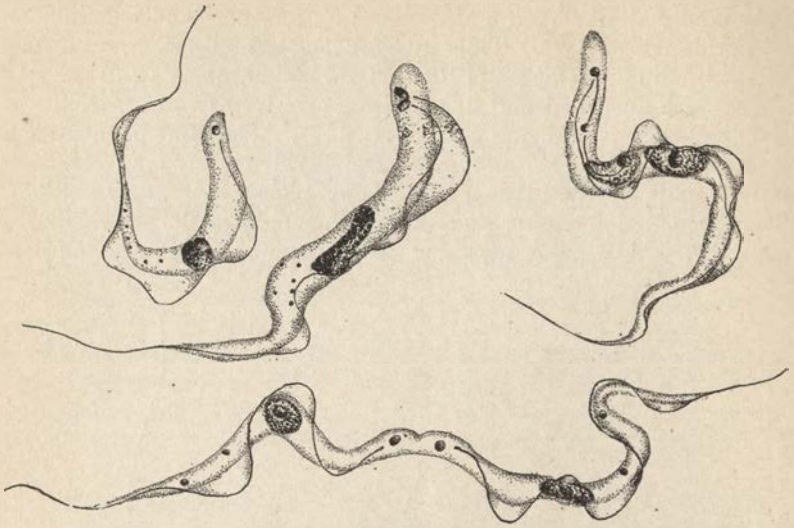

FIG. 97.—*Trypanosoma gambiense*, one cause of African sleeping sickness. Normal individual and successive stages in division of blepharoplast, nucleus and cell. (After Calkins.)

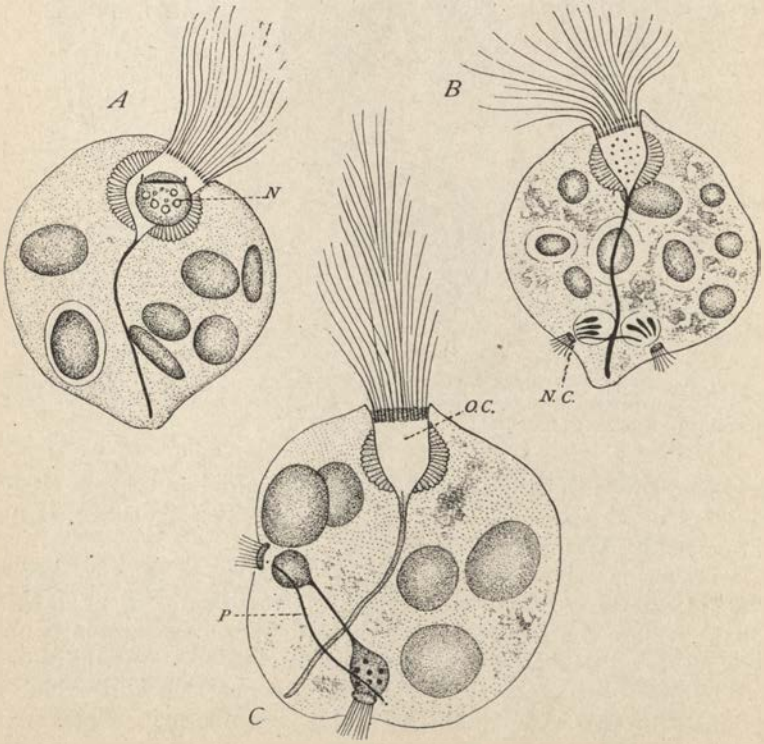

FIG. 98.—Division of *Lophomonas blattarum*. *A*, Nucleus leaving the old calyx, centrioles and paradesmose present; *B*, the nucleus at the posterior end of the cell, divided; *C*, development of daughter-calyces and bundles of flagella. (After Janicki.)

the calyx in which it normally lies, and moves with the spindle to the posterior end of the cell. The spindle takes a position at right angles to the long axis of the cell; chromosomes, probably eight in number, are formed and divided, and two daughter nuclei result, each of which is enclosed by a new calyx while new basal bodies and blepharoplasts apparently arise from the polar centrioles (Fig. 98, *B*, *C*). Thus the old kinetic complex, with the exception of the cytoplasmic centriole, is discarded and entirely new aggregates are formed.

Flagellates with shells or tests behave during division in different ways. In the majority of cases division occurs within the test; the daughter individuals leave the old test by way of the aperture and form new tests; in other cases the tests as well as the cell bodies divide, as in the Diniferida. As the apical and antapical poles are different in the Dinoflagellida division is followed by regeneration of the appropriate shell part that is missing.

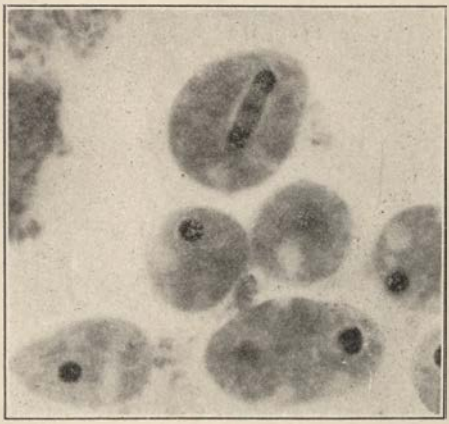

FIG. 99.—*Vahlkampfia limax*. Nucleus in upper cell in full mitosis (promitosis). (From Calkins.)

B. Division and Reorganization in the Sarcodina.—It is very questionable whether any rhizopod divides in the very simple manner described by F. E. Schultze for *Amœba polypodia*. The "limax" types indeed approach this simplicity (Fig. 99) but new discoveries are constantly at hand to indicate that these are not as simple as they have been described. Thus Arndt (1924) quite recently has given creditable evidence of the existence in a simple amœba, *Hartmannella* (*Pseudochlamys*) *klitzkei*, of a definite centrosome with centriole which is permanently extranuclear (Fig. 41, p. 85). At division of the cell the centrosome divides and the daughter centers with their centrioles, take positions at

the poles of the nuclear spindle which originates within the nucleus. The mitotic figure is thus made up of cytoplasmic elements, kinetic elements derived from the nucleus, and chromatin. A similar combination occurs in dividing Heliozoa. The original description of division of *Acanthocystis aculeata* by Schaudinn, a form possessing the characteristic central granule of the Heliozoa, has been considerably modified by later observations. According to Schaudinn the central granule or centroblepharoplast which is the focal point in the cell of the radiating axial filaments, divides to form an amphiaster (Fig. 100) which becomes the central spindle of a typical

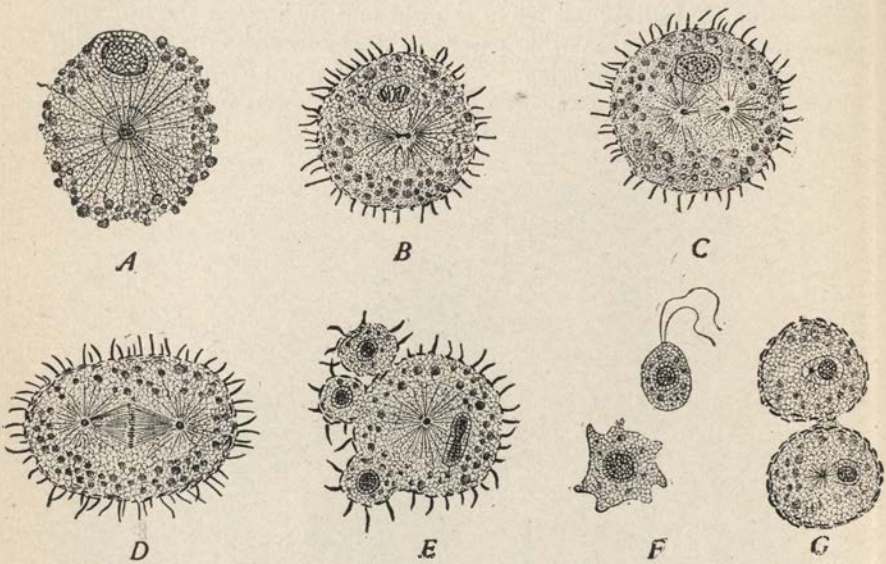

FIG. 100.—*Sphærastrum* and *Acanthocystis*. *A*, Vegetative cell of *Sphærstrum* with axial filaments focussed in a central granule (centroblepharoplast); *B*, *C*, *D*, division of central granule and spindle formation in *Acanthocystic aculeata; E*, *F*, formation of buds of same; *G*, exit of central granule from the nucleus of young cells. (After Schaudinn.)

mitotic figure. The more recent observations of Stern (1924) indicate that, as in the simpler amœba described above, the central granule of *Acanthocystis* behaves as a cytoplasmic centrosome, forming poles of a mitotic figure which is derived otherwise entirely from the nucleus. Individuals which have been deprived of their skeletons and membranes which afford resistance to the activities of the enclosed protoplasm, become "sprung," so to speak, and the unusual freedom from restraint results in a separation of the centrosomes from the remainder of the spindle which completes its division without further participation of the centrosomes (Fig. 101).

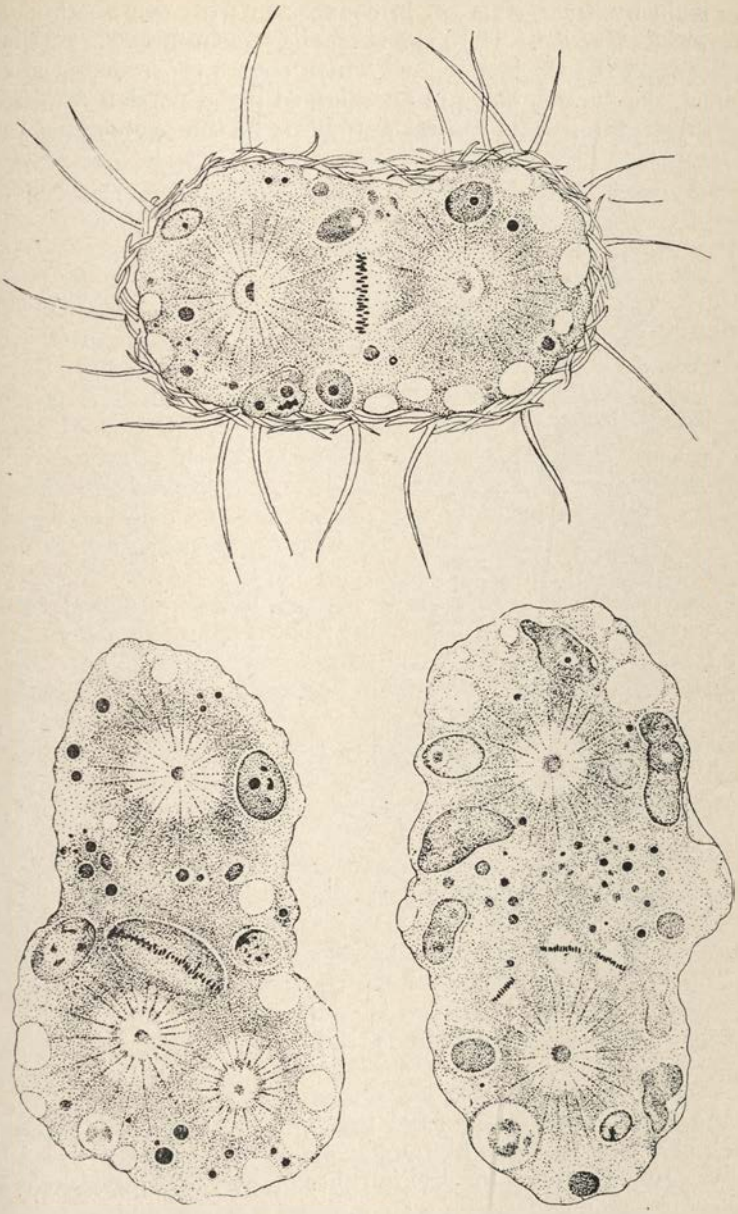

FIG. 101.—*Acanthocystis aculeata;* centroblepharoplasts disconnected from nuclear spindle. (After Stern.)

Schaudinn's description of division in Heliozoa was confirmed in the main by Zuelzer (1908) in connection with the aberrant form *Wagnerella borealis*. Here the axopodia-bearing portion of the cell is free from the silicious mantle which covers the remainder of the animal, the nucleus being in an enlarged pedal portion attached to the substratum. The central granule is in the geometrical center

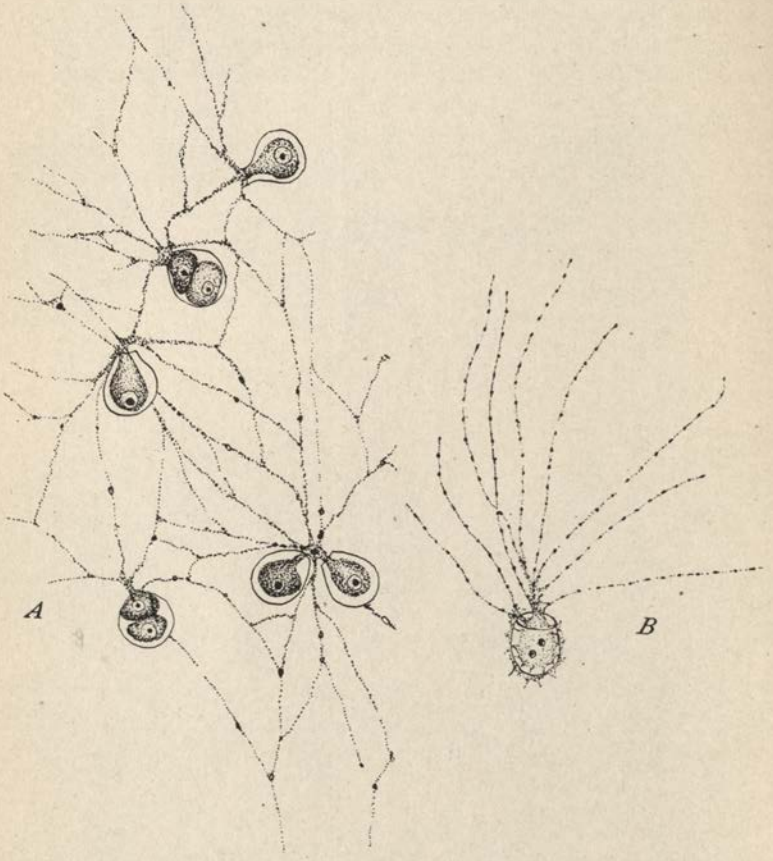

FIG. 102.—*Microgromia socialis* after Hertwig (*A*), and *Microgromia* sp. (*B*.) original.

of the "head" and is the focal point of the axopodial filaments. Each of the latter bears a granular enlargement similar to a basal body. In preparation for division these move centripetally toward the central granule forming a zone about it which divides with the division of the central granule. In the meantime the nucleus migrates from the other end of the body and with the spindle formed by the divided central granule forms the mitotic figure.

Complications in the division process accompany the presence of shells and tests. Where these are chitinous or pseudochitinous, they may also divide with the cell body (*Pseudodifflugia, Cochliopodium*). In other cases the individual divides within the shell, after which one of the daughter individuals moves out and forms a new shell, while the other one remains in the original test (*Microgromia socialis, Clathrulina elegans*, etc., Fig. 102). In most cases, however, a novel method of shell duplication found in no other division of the Protozoa, has been developed. This process, known as budding division, occurs throughout the group of the testate rhizopods and is well illustrated by the classical example of *Euglypha alveolata* first described by Schewiakoff (1888). Here after full growth following vegetative activity of the individual, the pseudopodia are drawn in; water is then absorbed whereby the protoplasmic density is greatly reduced and the volume increased. This is followed by a process resembling pseudopodia formation, the protoplasm emerging from the parent shell opening as a ball or dome which assumes the general form of the parent organism. A new membrane of pseudochitin is formed about the extruded mass and on it the silicious shell plates, preformed in the parent protoplasm, are now cemented. In some forms, *e. g., Arcella* species, the chitinoid membrane becomes the permanent shell of the organism, older shells becoming brown or reddish by coloring due to oxides of iron; in other forms as in the Difflugiinæ the chitinoid membrane is covered by foreign objects picked up and stored by the parent organism. In all cases of budding division after the budded individual is fully moulded, the nucleus divides and one-half passes into the protoplasm of the new shell. The connecting zone of protoplasm between the old and the new shell breaks out into pseudopodia and the two individuals separate (Fig. 10, p. 32).

The various types of foraminiferal shells, nodosarine, frondicularine and rotaline—may be interpreted as due to a similar budding division, but without actual separation of the parent and bud protoplasm, the type being dependent upon the density of the protoplasm at the time of protrusion from the shell mouth (Fig. 17, p. 38).

There is very little evidence of reorganization of the protoplasm at division in these rhizopods. The frequent withdrawal of pseudopodia and rounding of the body may be an indication of changes going on within, as in *Chlamydomyxa, Nuclearia*, etc., but even such questionable indications are absent in many cases of recent investigation (Belar, Stern, *et al.*), where reorganization, if it occurs at all, must be in the make-up of the protoplasmic and undifferentiated elements (see, however, *infra*, p. 484).

C. Division and Reorganization in Infusoria.—Here in the most highly differentiated forms of the Protozoa the processes of equal division are complex and the protoplasmic changes far-reaching.

With but few exceptions the division plane is through the center of the body and in a plane at right angles to the long axis of the cell. The externals of division are similar to division in other groups, with preliminary division of the plastids and nuclei and final division of the cell body. As in flagellates and some rhizopods the cup or test-dwelling forms divide within the parent cup, one of the daughter individuals migrating and forming a cup for itself. In some forms the daughter individuals remain and share the old house (*Cothurnia ingenita*).

Where a tightly-fitting cell-covering is present as in *Coleps hirtus*, it is divided transversely and the missing parts are regenerated by the daughter organisms (Fig. 65, *A*, *B*, *C*, p. 128). In some Infusoria as in the other groups, division in many cases is incomplete, the daughter individuals remaining attached end to end as in *Polyspira delagei* or *Haptophrya gigantea* (see chain building in *Ceratium vultur*, Kofoid). Or daughter individuals may remain attached by incomplete division of their stalks, thus giving rise to arboroid colonies of different types (Vorticellidæ mainly).

In some forms, probably in the majority of ciliates, there appears to be a definite and permanent division zone which indicates the future plane of division and which is not displaced even after diverse mutilations of the body. Thus if *Paramecium caudatum* is cut across either the anterior or the posterior end, the cell ordinarily does not regenerate more than a ciliated surface on the truncated end. It divides like a normal form the division plane, however, is not in the geometrical center of the mutilated cell, but in the geometrical center of the cell as it was before the cutting (Fig. 103). The same is true of *Uronychia transfuga* or *U. setigera* (Fig. 108). In daughter cells of dividing *Paramecium* the future division zones appear to be formed at an early period, and if a daughter cell is cut in such a manner that the geometrical center is destroyed without, however, destroying the nuclei, monsters of various types are produced indicating a complete upset of the organization (Fig. 103, *f-o*). In some cases, *e. g.*, *Frontonia leucas*, the geometrical center, or division zone, has a different physical appearance from the remainder of the cell (Popoff, 1908, also mentioned by Hance, 1917 as occurring in *Paramecium*), but in the majority of cases there is no morphological evidence of the plane of division during resting stages.

(*a*) **Evidence of Nuclear Reorganization.**—The two types of nuclei, macronucleus and micronucleus, complicate the nuclear phenomena at division. The macronucleus is more like a huge plastid of the cell with active functions in metabolism, while the micronucleus is generally interpreted as a germinal or racial nucleus, functioning at division and particularly at conjugation.

Reproduction of the macronucleus in the majority of ciliates is

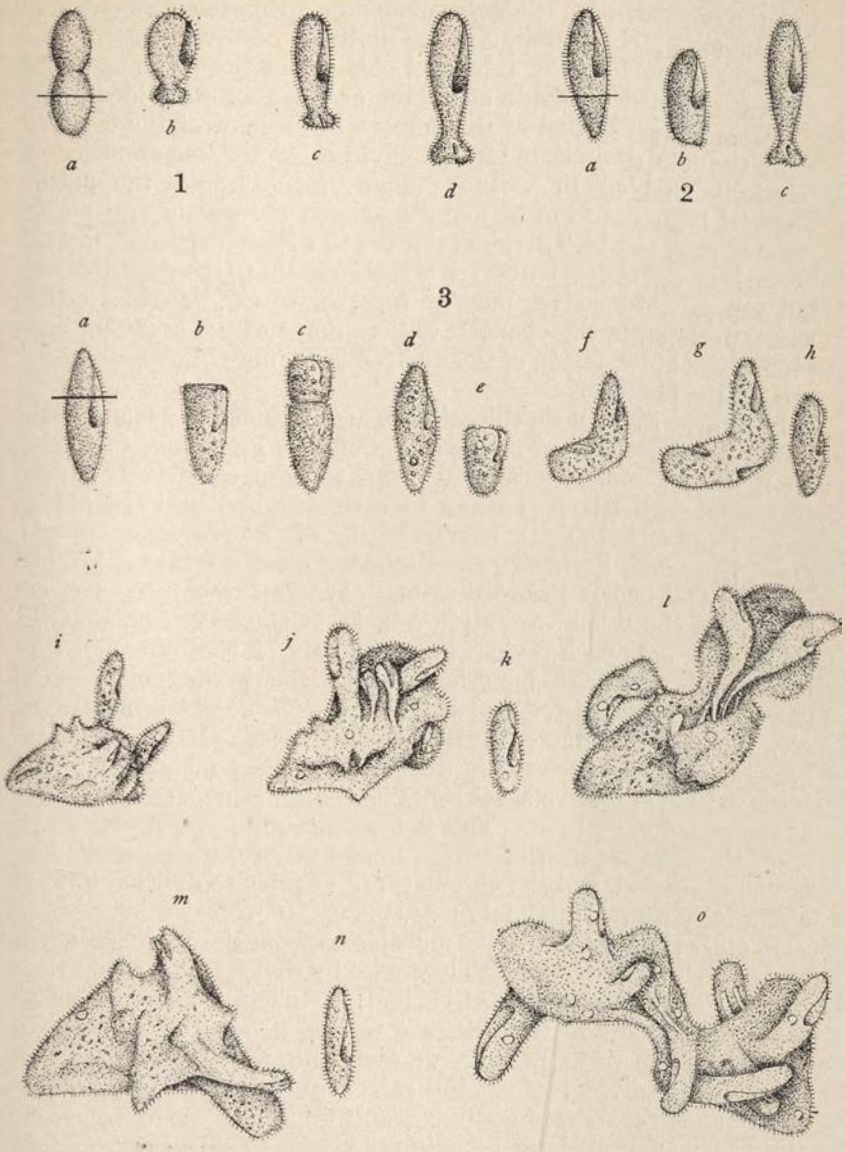

FIG. 103.—*Paramecium caudatum*, merotomy. 1, 2, and 3, different experiments the straight line indicating the plane of cutting; 3, the history of a monster; an original cell 3*a*, was cut as indicated; the posterior fragment (*b*) divided (*c*) into (*d*) and (*e*), the latter formed a monster (3, *f*,-*o*); enucleated individuals (*h*, *k*, and *n*) occasionally separated from the parent mass. (After Calkins.)

analogous to that of a plastid. Division is direct with only a few isolated cases showing evidences of spindle formation or of indefinite chromosomes. In preparation for division, however, there is evidence in many forms of profound changes in the make-up of the nucleus destined to divide and some of these afford evidence of a clear-cut reorganization of this important element of the ciliate.

In the less complicated types division of the macronucleus is relatively simple. In *Dileptus anser*, for example, the nuclear material is in the form of many scattered chromatin and plastin spheres, each of which divides prior to cell division (Fig. 58, p. 116). There is no equal distribution of this chromatin to the daughter cells but the daughter halves may go together to the daughter cell in whose protoplasm they happen to lie. Some of the granules, however, those in the region of the division zone, may be represented in each of the progeny.

In forms with a single ellipsoidal macronucleus as in many of the commoner types (*e. g.*, *Paramecium*, *Colpoda*, *Frontonia*, *Glaucoma*, etc.), the macronucleus simply elongates and constricts to form two equal portions, one passing to each daughter cell (Fig. 21, p. 53). Band-form nuclei characteristic of *Blepharisma*, *Spathidium*, *Didinium*, *Vorticella*, *Euplotes*, etc., condense into spheroidal or ellipsoidal bodies before dividing. Where two macronuclei are present in the usual vegetative cell, as in *Oxytricha*, *Stylonychia*, *Gastrostyla*, etc., each divides independently of the other but synchronously. As with band-form nuclei the beaded macronuclei likewise form short rods as in *Stentor*, *Spirostomum ambiguum*, etc., the beaded character in all cases being lost. Here the separate beads are usually enclosed in a common nuclear membrane which is constricted at intervals, the contained chromatin massing together at the period of division. This is the condition in *Uronychia transfuga*, also, the twelve to fourteen apparently separate macronuclei are all connected, and the chromatin fuses prior to division to form a relatively short ellipsoidal nucleus (Fig. 107).

In other types, however, the multiple macronuclei are independent and entirely disconnected. They arise by division and retain their independence during vegetative life. Thus in *Uroleptus mobilis* the eight or more macronuclei are formed as a result of a fourth division of the single parental nucleus from which they came. In preparing for division of the cell each of these eight nuclei of *Uroleptus* undergoes a remarkable transformation. A nuclear cleft (Kernspalt) appears in each, and in the cleft is a single large granule which reproduces by division. The major part of the nucleus lies below the cleft and is filled with densely staining chromatin; the other part lying above the cleft contains much less chromatin in the form of fine granules (Fig. 104). This latter part, together with the granules in the cleft, are thrown off and the chromatin contents

are distributed in the cytoplasm. When each of the nuclei is thus freed from its distal portion the eight remaining parts fuse together,

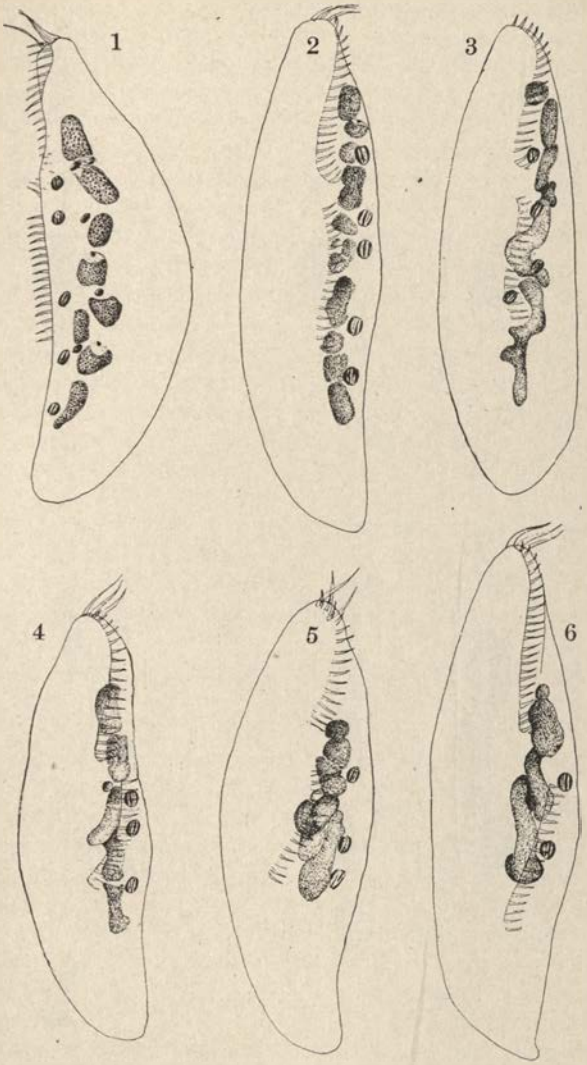

FIG. 104.—*Uroleptus mobilis.* Stages in the fusion of the macronuclei prior to cell division; micronuclei in mitosis. (After Calkins.)

forming first a long banded nucleus, and later, by condensation, a relatively small ellipsoidal and single nucleus. This divides twice or three times before the division of the cell is completed, the fourth

division always occurring after the daughter cells have separated (Fig. 105).

The micronuclei show no such complicated histories. If they are multiple in the cell there is no fusion, nor is there any elimination

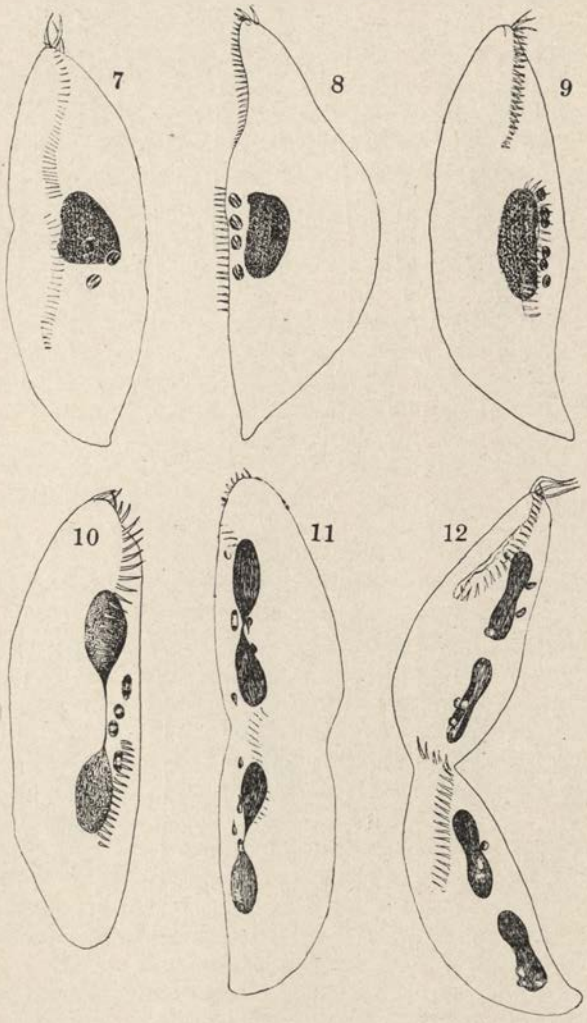

FIG. 105.—*Uroleptus mobilis.* Division stages after fusion of the macronuclei. (After Calkins.)

of micronuclear material. Each divides with the formation of an unmistakable but very minute, mitotic figure (Fig. 22, p. 57). They are all represented furthermore by daughter halves in each of the daughter cells.

b. **Evidence of Cytoplasmic Reorganization.**—Not only is there evidence of change in the cytoplasmic make-up at division through the distribution and absorption of nuclear material as in *Uroleptus mobilis*, but the entire cytoplasm shows other evidence at this period. In all ciliates there is a more or less clearly marked antero-posterior differentiation, the anterior part usually bearing the mouth and the more or less specialized motile organs for the capture of food or the directing of food currents, while the posterior part is usually much less specialized. Should such a specialized ciliate be cut through the center as Balbiani (1888) did for the first time, the two fragments would be different. The anterior fragment of a *Stylonychia* or *Uronychia*, for example, would retain the highly differentiated parts about the mouth while the posterior part would be relatively undifferentiated. The finer organization or genotype, however, is represented by all of the protoplasm of the cell, and that organization has the ability under proper stimulation, of forming all of the differentiated parts of the entire adult organism. By regeneration, therefore, such a cut individual replaces the characteristic structures of the posterior end by the anterior fragment and the characteristic structures of the anterior end by the posterior fragment (Fig. 108). By their usual method of transverse division the ciliates have quite a different inheritance than do flagellates which divide longitudinally. In the latter the highly differentiated anterior ends and the less differentiated posterior ends are equally divided so that the daughter cells have a like inheritance (p. 209).

The processes through which the ciliate cell passes during division indicate that the organism is restored to a generalized condition practically equivalent to an encysted cell. Except for the cytostome the entire array of complex cortical organs is withdrawn and a new set is formed from the cortical protoplasm. This significant process first described by Wallengren (1900), later by Griffin (1910) in hypotrichous ciliates, has been observed in many forms and is probably characteristic of the entire group. It is most clearly established in the Hypotrichida where the highly specialized and conspicuous motile organs furnish suitable material for study. According to Wallengren's description the membranelles of the adoral zone slowly decrease in length as the process of absorption continues and at the same time minute buds of protoplasm appear at the bases of these disappearing membranelles. These buds grow *pari passu* with the dwindling motile organs until finally the latter are entirely absorbed and the buds have developed into functional membranelles. In the same way each cirrus is replaced by a new growing bud quite regardless of the position in anterior or posterior half. Undulating membranes are similarly withdrawn and replaced by new ones so that the young cells formed by division of the meta-

morphosing parent cell receive a full set of new motile organs commensurate with the size of the young organisms. The phenomenon is very striking in forms with giant cirri such as the jumping types of Euplotidæ—*Diophrys* or *Uronychia*. In the latter genus the great posterior cirri are the most conspicuous organs of the cell (Fig. 107). The buds which are to grow and replace them are apparent before there is other external evidence of the approaching division and even before the nucleus has concentrated into its division form. At the same time similar buds appear in the division zone, that which is destined to form the giant hooked cirrus appears first and is always larger than the others which appear one after the other according to ultimate size. Owing to their minute size it has not been determined whether or not the individual cilium is withdrawn in like manner and replaced by new ones. In some, at least, according to the observation of MacDougall on *Chilodon uncinatus* (1925) such substitution does take place and it is quite probable that it is universal. The interesting experiments of Dembowska (1925) show that removal of a single cirrus of *Stylonychia mytilus* causes regeneration of the entire motile apparatus, but no such result follows extirpation of any body region that is free from cirri or cilia.

The phenomenon is obviously analogous to the absorption and renewal of flagella in the flagellates. Whether or not there is a similar division of the basal bodies of the cilia has not been fully established.

Other evidence of protoplasmic reorganization at division is furnished by the history of some of the functional metaplastids of the cell. Trichocysts are apparently handed down without change (Fig. 21, p. 53), but there is good evidence that the more complicated aggregates of trichites are absorbed and replaced by new ones. This is the case for example in the Chlamydodontidæ, where the complex oral baskets are replaced by new ones at each division Enriques, Nägler, MacDougall, *et al.*, (Fig. 106).

From this brief survey it is quite evident that far-reaching changes of the protoplasmic organization take place at periods of division. Both nuclei and cytoplasm are necessary but the micronucleus apparently may be lost without destroying the power of the cell to divide. Emicronucleate races of ciliates, arising possibly through defective reorganization and division after conjugation (see Moore, 1924), have been maintained in culture for many generations by division, although they are ultimately lost (see Chapter X). On the other hand, the power to regenerate is connected in some manner with the micronucleus. Thus young cells of *Uronychia transfuga*, when transected with a scalpel, will regenerate only that fragment which contains the micronucleus (Calkins, 1911, Fig. 108; Young, 1923). In old cells, however, both fragments regenerate regardless

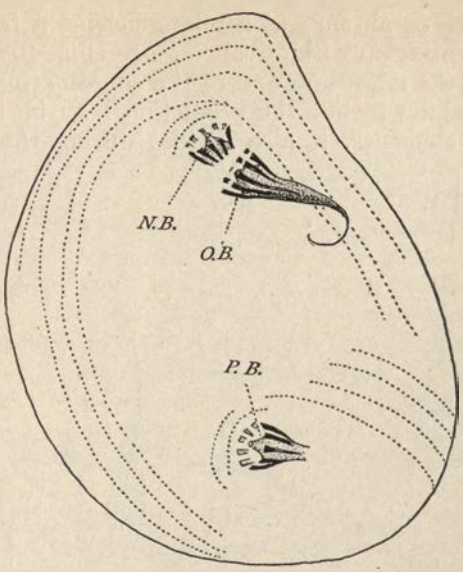

FIG. 106.—*Chilodon uncinatus.* New mouth and basket replacing the old ones prior to cell division. (*N.B.*) New mouth and basket; (*O.B.*) old mouth and basket before degeneration and disappearance; (*P.B.*) new mouth and basket for the posterior individual after division. (After MacDougall.)

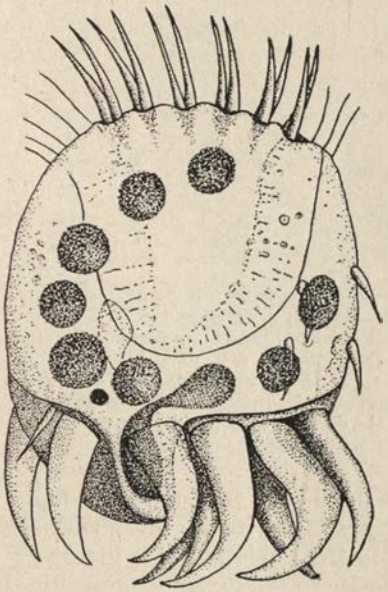

FIG. 107.—*Uronychia transfuga* with giant cirri, membranelles used in swimming, ten macronuclear segments, and single micronucleus. (After Calkins.)

of the presence or absence of a micronucleus, a fact indicating a change in organization with advancing age (Fig. 108, 5).

The fate of the motorium and of the coördinating fibrils at division is still unknown, but the prediction may be made that, like other kinetic elements, it also divides during the reorganization

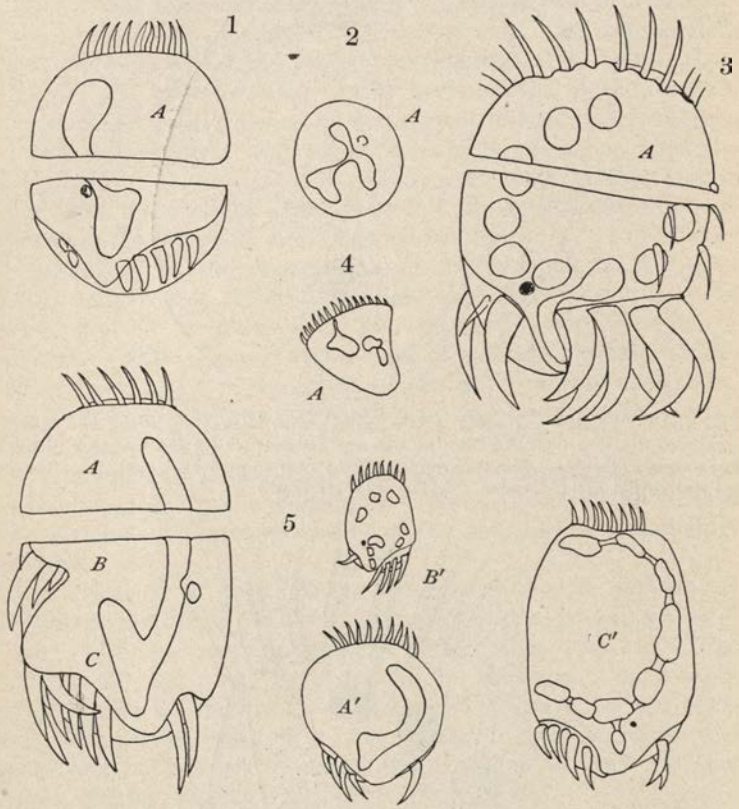

FIG. 108.—*Uronychia transfuga*, merotomy and regeneration. 1, cell immediately after division, cut as indicated; 2, fragment *A* of 1, three days after the operation; no regeneration; 3, cell cut five hours after division; 4, fragment *A* of 3, three days after operation, no regeneration; 5, cell cut at beginning of division as indicated into fragments *A*, *B*, and *C*; *A′*, *B′*, *C′*, fragments *A*, *B* and *C*, twenty-four hours after the operation; fragment *A* regenerated into a normal but emicronucleate individual (*A′*); *B*, *C* divided in the original division plane forming a normal individual (*C′*) and a minute but normal individual (*B′*). (After Calkins.)

process. It is a significant fact that the peristome and the peristomial organs appear first in the more specialized anterior half of the ciliate cell, and from this position gradually shift to the region immediately posterior to the division zone (Fig. 105). In *Vorticella* according to Bütschli (1888) after Fabre, the peristome and adoral zones are reversed in the daughter cells.

II. UNEQUAL DIVISION (BUDDING OR GEMMATION).

In reproduction by budding or gemmation, one or more minute fragments of the cell are produced by unequal division of the organism. Parent and offspring are thus distinguished, their relative sizes varying in different cases. In many instances both parent and offspring continue to live after such reproduction. In many other instances the residual parental protoplasm is no longer able to carry on metabolic activities and dies. Illustrations of both types abound in all groups of the Protozoa, the buds being formed either on the periphery of the parent in so-called exogenous budding, or within the protoplasm of the parent in so-called endogenous budding. The minute cells that are formed by budding always contain a portion, sometimes one-half, of the nuclear structures of the parent and may develop asexually into organisms similar to the parent, or they may be differentiated as gametes requiring fertilization before development.

A. Exogenous Budding.—In Mastigophora such reproduction by unequal division is uncommon, but may be found in some of the simpler types of Chrysomonadida (*Pedinella hexacostata*, *Cyrtophora pedicellata*, *Palatinella cyrtophora*, etc. (Fig. 94, p. 200). Here a portion or portions of the oral region within the circlet of tentacles appear as club-shaped or spheroidal protuberances which break way from the parent and develop independently.

In other cases of unequal division amongst flagellates the parent cell dies after giving rise to numerous offspring. Thus in *Noctiluca miliaris* many bud nuclei are formed by repeated mitotic divisions of the nucleus, one division following another so quickly that full mitotic figures may be seen connected by the, as yet undivided, nuclear strand of the preceding division (Fig. 109). Several hundred buds are formed as protuberances on the surface of the cell, each with a compact nucleus. These buds when ready to leave the parent have the structure of a dinoflagellate with a rudimentary tentacle, transverse furrow and a flagellum (see Kofoid, 1920).

In Sarcodina unequal division similarly results in death to the parental protoplasm after the buds are given off, but in many such cases the observations are not convincing. Thus Schaudinn (1903) described exogenous budding in *Endamœba dysenteriæ* (*histolytica*) as a normal method of reproduction, but later observers interpret such stages as evidence of degeneration of the parasite, pathological rather than cyclical (see Darling, Dobell, Cutler). Quite similar budding phenomena described by Schaudinn for the *Leydenia* form of *Chlamydophrys stercorea*, and by Hogue for the oyster parasite *Endamœba calkensi* and for *Endamœba patuxent* are subject to the same criticism. In all of these cases there is no division of the nucleus but collections of chromidia function as the

nuclei of the so-called buds (Fig. 63). In *Councilmania lafleuri*, Kofoid and Swezy (1921) which is considered an aberrant form of *Endamœba coli* by some authorites, the phenomenon of exogenous budding is quite different from so-called budding in the amœbæ mentioned above. Here according to Kofoid and Swezy, the nucleus divides three or more times to form from eight to sixteen nuclei which, enclosed in buds of cytoplasm are successively pinched off from the surface of the amœba (Fig. 110).

In *Acanthocystis aculeata* according to Schaudinn (1896) and in *Wagnerella borealis* according to Zuelzer (1909) the nucleus of the cell divides one or more times by simple constriction and without the formality of mitosis or participation of central granule. The minute nuclei thus formed wander to the periphery of the cell where

FIG. 109.—Exogenous buds of *Noctiluca miliaris*. (After Robin.)

they are pinched off in minute cells. In *Acanthocystis* these buds form minute amœbæ which after four or five days of activity settle down and metamorphose into young Heliozoa. The buds have no central granule but during metamorphosis a kinetic element emerges from the nucleus and this becomes the central granule of the adult *Acanthocystis* (Fig. 100, p. 214). In *Wagnerella borealis*, according to Zuelzer, the buds which are formed in a similar manner are flagellated, but her description in other respects follows that of Schaudinn.

In Infusoria, particularly in Suctoria, exogenous budding is not uncommon. In Ciliata it is comparatively rare and limited apparently to the Spirochonidæ. In *Spirochona gemmipara* according to Hertwig a swelling appears at one side of the base of the peculiar

funnel-like peristome. The nucleus divides equally, one-half passing into the swelling which, with only partial peristomial development, breaks away from the parent and then completes its peristomial differentiations.

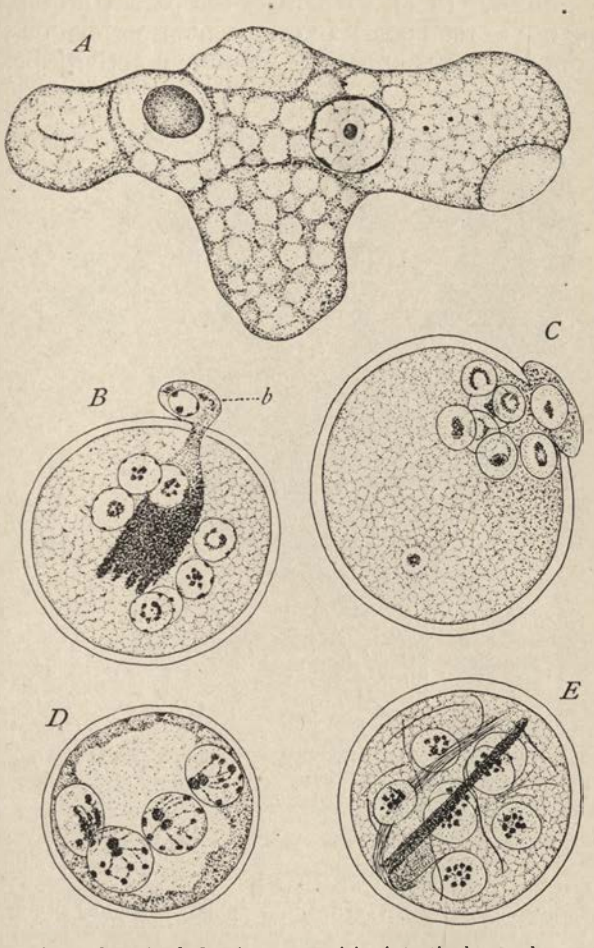

FIG. 110.—*Councilmania lafleuri*, a parasitic intestinal amœba. *A*, normal, vegetative individual; *B*, to *E*, encysted individuals and formation of eight endogenous buds which escape one by one (*B*, *C*). (After Kofoid and Swezy.)

In Suctoria similar exogenous buds, either single or multiple, are formed from the oral extremity of the cell (Fig. 111). Such buds are dissimilar to the parent which they come to resemble only after a period of metamorphosis and development.

In Sporozoa with the exception of some Cnidosporidia, exogenous budding is limited to unequal division in gamete-forming processes.

Thus in Gregarinida and in microgametocytes of Coccidiomorpha the nucleus of the cell undergoes several divisions, the final products arranging themselves about the periphery from which they become nuclei of variously formed gametes budded out from the surface (Fig. 179, p. 420). In all such cases the parent protoplasm dies after giving rise to the buds. In some Cnidosporidia, on the other hand, budding processes appear to be normal activities carried on

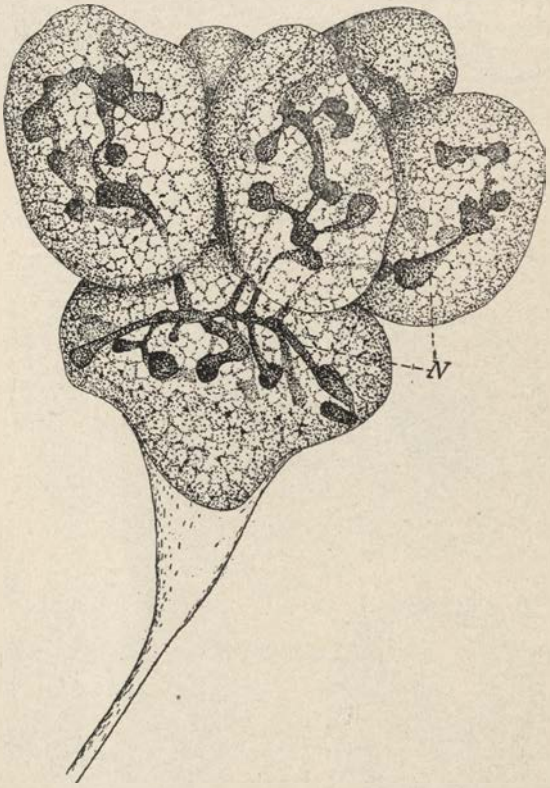

FIG. 111.—*Ephelota bütschliana*, a suctorian. Budding individual with five exogenous buds. *N*, branching macronucleus. (After Calkins.)

during the vegetative life of the organisms. According to Cohn (1895) large numbers of buds, each containing several nuclei, may be formed from the periphery of *Myxidium lieberkühni*. The phenomenon appears to be an exaggeration of the peculiar process of division termed plasmotomy by Doflein, whereby a multinucleated cell divides spontaneously into two more or less equal parts as in *Chloromyxum leydigi* according to Lühe and Doflein, or into several parts, as in the Coccidian *Caryotropha mesnili* and *Klossiella*

muris and termed "schizontocytes," or "cytomeres" by Siedlecki (1902).

B. Endogenous Budding.—This type of unequal division is not so widely distributed amongst Protozoa as is exogenous budding and is apparently not represented at all in flagellated forms. It does occur, however, in all of the other groups.

In Sarcodina endogenous budding has been described mainly in connection with the testate rhizopods. In *Centropyxis aculeata* according to Schaudinn (1903) it leads to gamete formation, but in *Arcella vulgaris*, according to Swarzewski (1908) and Elpatiewsky (1909) it is a form of asexual reproduction.

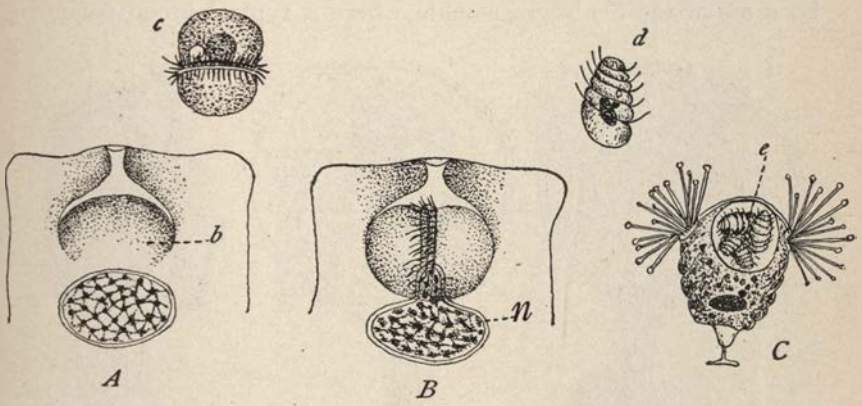

FIG. 112.—Endogenous budding in Suctoria. *A*, *B*, two stages in the formation of a bud (*b*) and (*c*), of *Tokophrya quadripartita*; *C*, *Acineta tuberosa* with endogenous buds (*e*) and (*d*). (From Calkins after Bütschli.)

In Infusoria internal budding is characteristic of many types of Suctoria, but is apparently not represented in the Ciliata. In the simplest cases the budding area at the anterior end becomes internal by insinking of the anterior surface and constriction of the body walls on all sides, so that the reproducing area is enclosed by living protoplasm which thus becomes a potential brood chamber within which the buds develop. Such buds may be single, as in *Tokophrya quadripartita* (Fig. 112 *A*, *B*), or multiple as in *Metacineta* (Fig. 112, *C*), and are always provided with cilia either as girdles or otherwise. Through the activity of these cilia the buds swim freely about in the brood chamber until they finally emerge through a "birth-pore" and after a variable period as free swarmers or as parasites in other Infusoria, they develop into adult forms of Suctoria. Cilia in Suctoria are thus confined to the embryonic stages and their various arrangements on the buds of different species recall the types of ciliation in the other branch of the Infusoria. In

one genus (*Hypocoma*) the embryonic cilia are retained throughout life.

A biologically interesting phenomenon of internal budding is described by Collin (1911) in the case of *Tokophrya cyclopum*. Here a brood pouch is formed by the cortical protoplasm within which the rest of the protoplasm becomes metamorphosed into a single bud with cilia. When mature this bud leaves the parent membrane on its old stalk and swims off as an embryo (Fig. 113).

In Sporozoa endogenous budding is manifested in a number of different ways. In some it is apparently a method of asexual reproduction, in others it is associated with gamete formation or with sporulation. Asexual reproduction by internal budding is illustrated by some of the Schizogregarinida where a typical brood pouch is

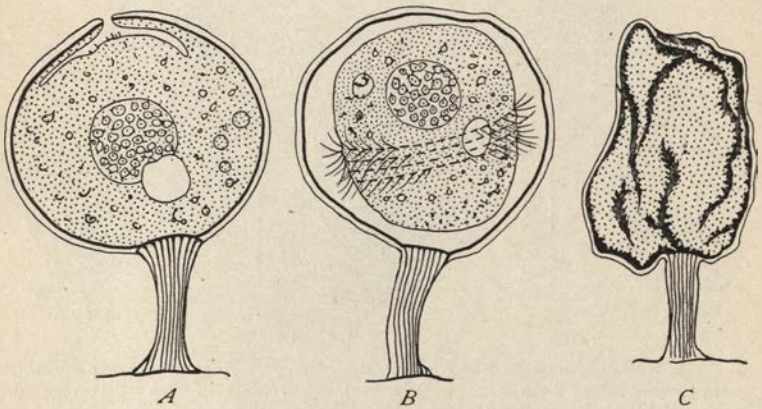

FIG. 113.—*Tokophrya cyclopum*, the entire cell, except the membrane, is used in the formation of a single bud which develops cilia (*B*) and swims off leaving the old membrane to shrivel up on its stalk (*C*). (After Collin.)

formed through which the internal buds escape through a birth opening as in Suctoria. In *Eleutheroschizon dubosqui*, according to Brasil (1906), the nucleus divides repeatedly until many are formed (Fig. 114, *A–D*). Each is then surrounded by a small portion of the parent protoplasm cut off from the rest of the cell. The central portion becomes vacuolated and opens to the outside, the agamonts making their way through the opening, leaving the remnants of the parental protoplasm to degenerate. Similarly in *Schizocystis sipunculi*, Dogiel (1907) described the formation of a brood pouch becoming filled with agamonts derived by internal budding from the parent protoplasm (Fig. 114 *E–G*). Gametes formed by internal budding are described by Leger (1907) in connection with the life history of *Ophryocystis mesnili*. Here after two "maturation" divisions of the nucleus in each of the gamonts

united in pseudoconjugation, a single free cell is formed in each gamont by internal budding (Fig. 115). Each bud here is a gamete

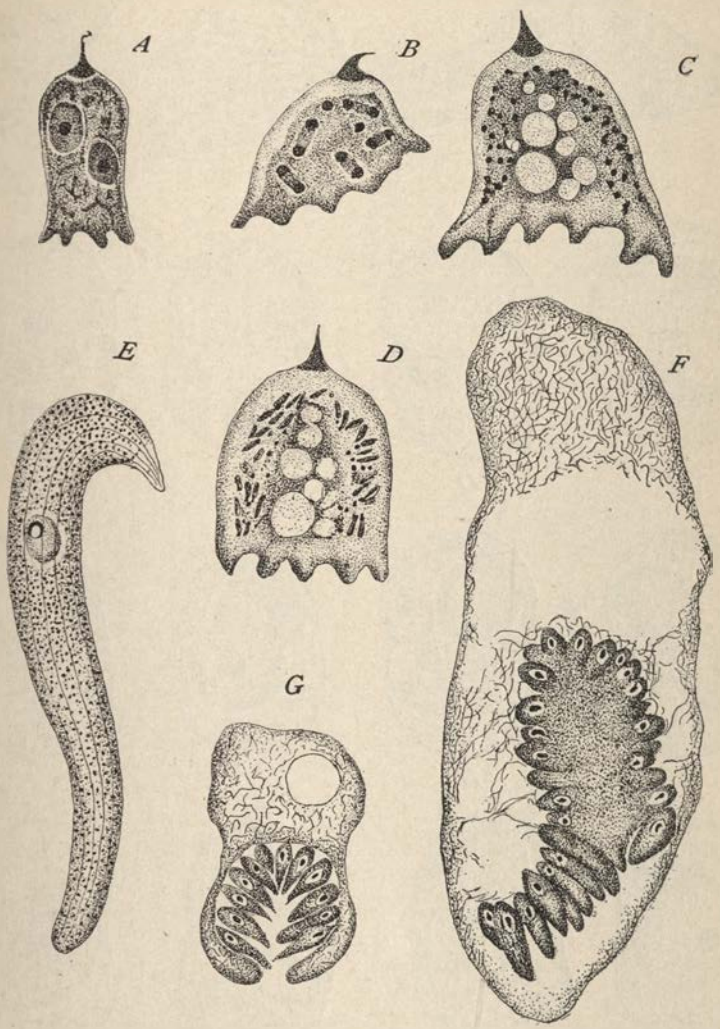

FIG. 114.—Endogenous budding in Gregarinida. *A* to *D*, *Eleutheroschizon dubosqui* and formation of endogenous agametes. (After Brasil.) *E* to *G*, *Schizocystis sipunculi* and similar formation of agametes. (After Dogiel.)

and the zygote is formed by union of the two in the parental brood chamber.

The phenomena of internal budding in the amœboid Myxosporidia of the Cnidosporidia, are still different in character and fate of the

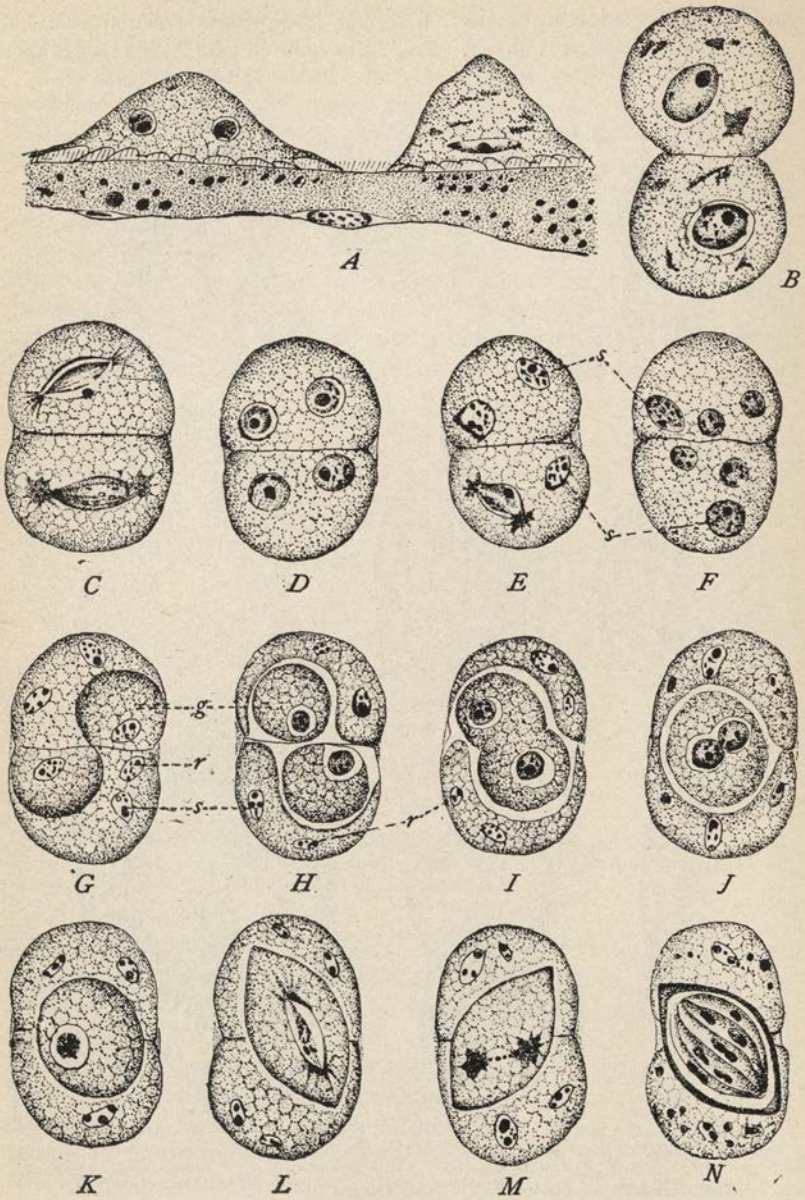

FIG. 115.—Gamete formation and fertilization in *Ophryocystis mesnili*. *A*, two individuals attached by processes to ciliated cells of a Malpighian tubule of *Tenebrio mollitor; B*, union of gamonts in pseudoconjugation; *C*, *D*, *E*, probable meiotic divisions of nuclei of the two gamonts; *G* to *K*, formation of two gametes and their union in fertilization; *L* to *N*, metagamic divisions resulting in eight sporozoites in the single sporoblast. (After Léger.)

buds. Here in the endoplasm local islands of protoplasm are quite separated from the surrounding protoplasm of the parent. Such islands, called pansporoblasts by Gurley (1893) or internal "cells"

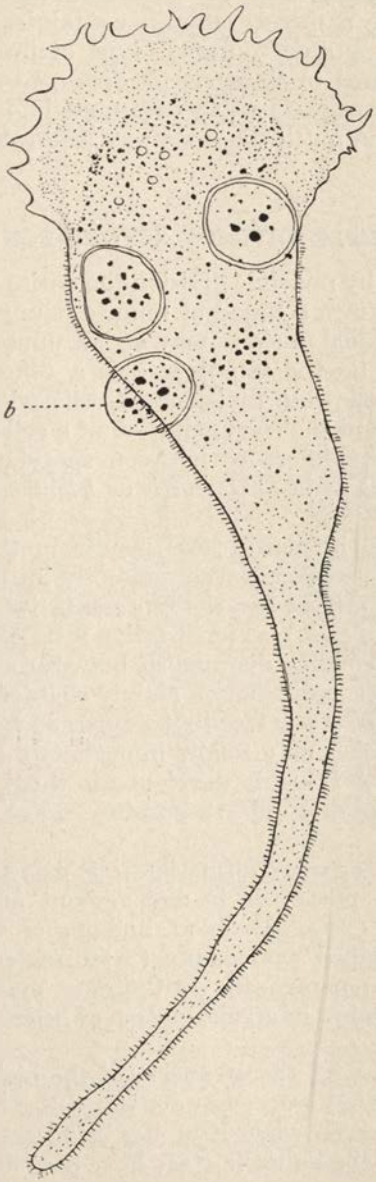

FIG. 116.—Internal buds or "gemmules," *b*, of *Sphærospora dimorpha*, a myxosporidian. (After Davis.)

by Davis (1916) are specialized reproductive centers in each of which one or more sporoblasts are formed. In the same living parent organism internal buds in various stages of maturity may be present and in some cases the amœboid parent organism may ultimately become a mere cyst wall containing large numbers of encysted young. A quite different type of internal bud called a "gemmule" is formed in *Sphærospora dimorpha* according to Davis (1916). These correspond to the agamont buds of the gregarines and leave the cell in much the same way as do the buds of *Councilmania* (Fig. 116).

III. MULTIPLE DIVISION (SPORE-FORMATION).

In reproduction by multiple division the entire protoplasm breaks up simultaneously into a brood of minute young, a mere fragment with perhaps a residual nucleus, may be left unused. Although the end-product may be the same there is a difference in principle between rapidly following divisions of cells within a cyst (as in *Colpoda cucullus*) and the fragmentation of a cell into many minute cells. There is less difference between sporulation and multiple endogenous budding as in *Schizocystis* or *Eleutheroschizon* described above.

Multiple division in many cases results in the formation of a brood of smaller cells which develop directly into organisms similar to the parent. In other cases the representatives of the brood are differentiated as gametes, and fertilization is necessary before development begins. We thus distinguish between sexual and asexual generations of spores, a distinction mainly characteristic of parasitic forms, but typical of many free-living types as well. In still other cases multiple division may follow immediately after fertilization, a phenomenon which is highly developed in the Sporozoa where the ultimate products of division—sporozoites—have a renewed potential of vitality.

Multiple division or spore formation thus may occur either in the agamont (asexual) phase, or in the gamont and zygote phases (sexual) of the life cycle. Division, budding or sporulation in the asexual phase is called agamogony (=schizogony); in the sexual phase gamogony (=sporogony). In the great majority of Protozoa the two phases together in an alternation of generations, make up a complete life history.

In Mastigophora with the exception of the highly differentiated Phytomastigida, sexual processes have in no case been safely established, multiple division when it occurs being agamogony. Many of the Euglenida in the Palmella stage have been described as giving rise to a multitude of spores, but such cases are more probably examples of repeated cell division under the protection of cyst

membranes as is the case in numerous Dinoflagellida, in *Chlorogonium euchlorum* (Fig. 117), *Phacotus lenticularius*, etc. In animal flagellates, however, particularly the parasitic forms, a highly characteristic method of multiple division is widely distributed. Here in certain phases or under conditions not yet well understood, trypanosomes, trichomonads, lophomonads and other parasitic flagellates undergo a process of asexual sporulation to which the specific term "somatella-formation" has been applied. It is well described by Minchin and Thompson (1915) in the case of *Trypanosoma lewisi* (Fig. 118) as follows:

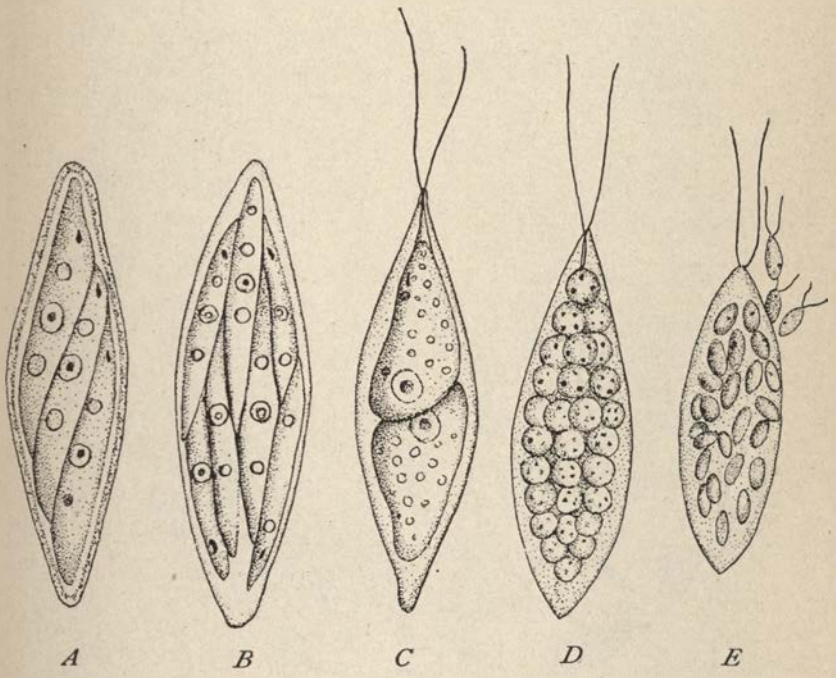

FIG. 117.—*Chlorogonium euchlorum*, formation of gametes. *A*, *B*, macrogametocyte forming eight macrogametes; *C*, *E*, *D*, microgametocyte forming microgametes. (From Doflein after Stein.)

"The parasites when taken up by the flea (*Ceratophyllus fasciatus*) pass with the ingested food into the stomach (mid-gut) of the insect. In this part they multiply actively in a peculiar manner, not as yet described in the case of any other trypanosome in its invertebrate host; they penetrate into the cells of the epithelium, and in that situation they grow to a very large size, retaining their flagellum and undulating membrane, and exhibiting active metabolic changes in the form of the body, which in early stages of the growth is doubled on itself in the hinder region, thus becoming pear-shaped

or like a tadpole in form, but later is more block-like or rounded. During growth the nuclei multiply, and the body when full-grown approaches a spherical form, and becomes divided up within its own periplast into a number of daughter individuals, which writhe and twist over each other like a bunch of eels within the thin

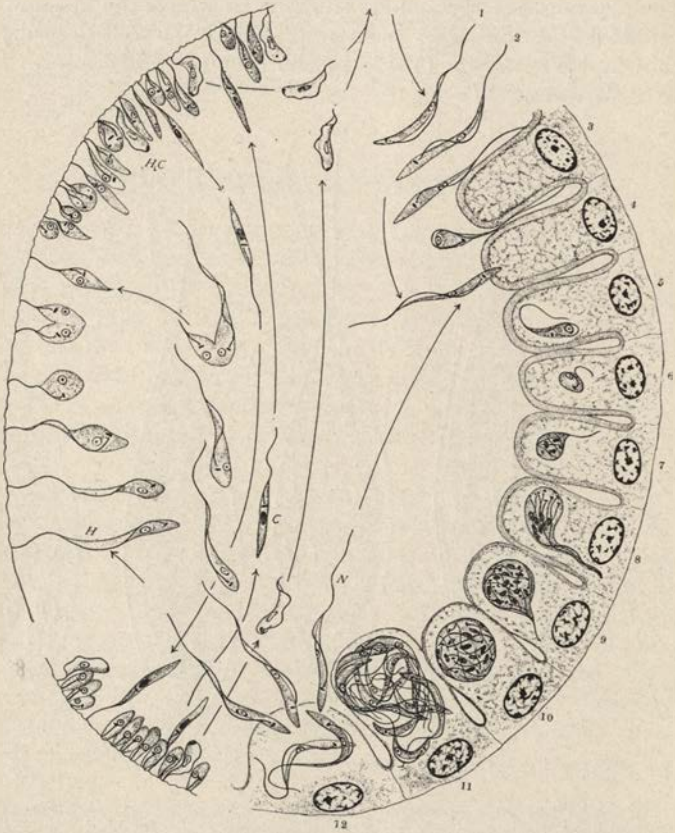

FIG. 118.—*Trypanosoma lewisi*. Cycle in the rat-flea *Ceratophyllus fasciatus*. 1, 2, blood trypanosomes entering the stomach; 3, 4, entering epithelial cells; 6–10, intracellular somatella-formation; 11, 12, adult trypanosomes leaving cell; *N*, young trypanosomes repeating intracellular phase; *C*, Crithidial forms; *H*, haptomonads reproducing by division. (After Minchin and Thompson.)

envelope enclosing them (Fig. 118, 11). When this stage is reached, the flagellum, which hitherto had been performing active movements and causing the organism to rotate irregularly within the cell, disappears altogether, and the metabolic movements cease; the body becomes almost perfectly spherical, and consists of the periplast envelope within which a number of daughter trypanosomes

are wriggling very actively; the envelope becomes more and more tense, and finally bursts with explosive suddenness, setting free the flagellates, usually about eight in number, within the host cell (Fig. 118, 12). The products of this method of multiplication are full-sized trypanosomes, complete in their structure, and differing

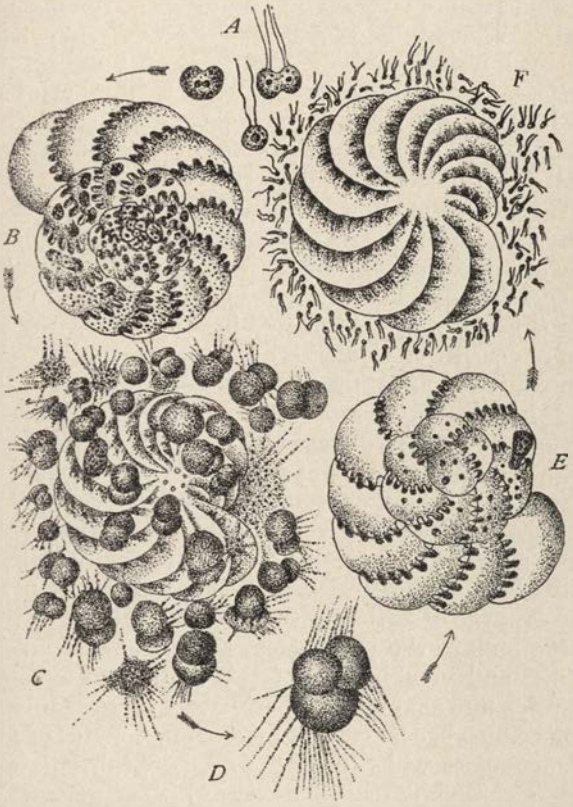

FIG. 119.—*Polystomella crispa*. *A*, zygote, (*A*) develops into an organism with a microsphæric type of shell (*B*) in which the nucleus divides by mitosis until many nuclei are present which form chromidia. The protoplasm fragments into reproductive bodies or agametes, each having several granules of chromidia (*C*). Each agamete develops into an adult with a macrosphæric-type of shell (*D.E.*): when adult these fragment into hundreds of flagellated gametes (*F*) which fuse in fertilization and so complete the cycle. (From Lang and Schaudinn.)

but slightly in their characters from those found in the blood of the rat. They escape from the host-cell into the lumen of the stomach." (loc. cit. p. 299).

Similar multiple division phases have been described for *Trypanosoma cruzi* (Chagas, Hartmann), for *Eutrichomastix serpentis*, and *Tetratrichomonas prowazeki* (Kofoid and Swezy), *Lophomonas blattæ*

(Janicki) and others. In these cases, as in *Trypanosoma lewisi*, the number of individuals formed is usually eight.

In Sarcodina there is a typical alternation of generations combined with multiple division best illustrated in the Foraminifera. According to the independent observations of Schaudinn (1903) and Lister (1905) the zygote develops into an agamont characterized by an initial central chamber of relatively minute size (microsphæric shell, Fig. 119, *B*). When fully grown the chromidia-laden protoplasm breaks up by multiple division into a great number of amœboid agametes (pseudopodiospores) each with a number of chromidial granules which fuse to form a nucleus. Each agamete develops into a gamont or individual of the sexual phase, characterized by a large initial central shell-chamber (macrosphæric shell Fig. 119, *D*, *E*). When these gamonts are mature, they also break up by multiple division into myriads of flagellated gametes (flagellispores, *F*). These are isogametes which fuse two-by-two, forming zygotes and these zygotes repeat the cycle by developing into microsphæric individuals (Fig. 119, *A*). Similarly in *Arcella vulgaris* there is an alternation of generations which is even more complicated than that of the Foraminifera according to the descriptions of Swarczewsky (1908) and Elpatiewsky (1909). A zygote (amœbula) develops into a typical adult *Arcella* agamont. This reproduces by agamogony in no less than four ways if these observers are correct.

A first method is by exogenous budding whereby agametes (amœbulæ) are liberated to develop again into agamonts. Another method is by multiple endogenous budding whereby many agametes are formed each of which develops into an agamont. A third method involves the desertion of the parent shell and of the primary nuclei by the bulk of the protoplasm and secondary nuclei formed by chromidia, and breaking up of this mass into agametes which likewise develop into agamonts. Ultimately these agametes develop into gamonts which become either macrogametocytes or microgametocytes, or gamonts which conjugate as do the ciliates with an interchange of chromidia (chromidiogamy). The macrogametocytes by multiple division give rise to macrogametes, and microgametocytes to microgametes. A macrogamete is fertilized by a microgamete and the resulting zygote repeats the cycle.

Multiple division is safely established for a number of Radiolaria although it is not yet determined whether the products are agametes or gametes. In many cases the flagellated swarmers which are thus formed by one individual are large while those formed from another individual are smaller. This has led to the view that the swarmers are anisogametes, but actual fertilization has not been safely established. They are formed from the materials of the central capsular protoplasm, which at first uninucleate, becomes multinucleate by repeated divisions of the nucleus. Comparatively

little cytological work has been done on these forms which offer a promising field for further research. According to Brandt (1885) the nuclear material is distributed about the endoplasm in the form of many clumps of chromatin which later become vesicular nuclei and undergo mitotic divisions. Hertwig (1879) describes the nucleus of *Acanthometra* as composed of a large endosome and a massive peripheral zone of chromatin which metamorphoses into a great number of small nuclei. In *Aulacantha scolymantha* according to Borgert (1900) the great primary nucleus gives off minute chromatin vesicles until the entire substance of the original nucleus is thus distributed in the endocapsular plasm and these become minute nuclei which now divide by mitosis. Ultimately the central capsule is dissolved, the phæodium disappears and the protoplasm breaks up into many small spheres each with several nuclei. Differences in these spheres indicate the later differences in the resulting swarmers. A somewhat similar history has been described for the giant nucleus of *Thalassicola*, but despite the observations of Brandt (1885) Hartmann and Hammer (1909), Huth (1913), Moroff (1910) and others, the significance of the peculiar processes is not clear. A rather unusual phenomenon is described by Haecker (1907) in *Oroscena regalis*. Here the huge single nucleus of the central capsule divides into two nuclei of which one remains as a functional nucleus of the organism, the other is interpreted as giving rise to gametocyte nuclei. There is also some evidence, not conclusive indeed, that an alternation of generations occurs, somewhat as in Foraminifera. Some types give rise by multiple division to isospores, *e. g.*, *Aulacantha*, which are biflagellated cells with characteristic crystalloid structures interpreted by Brandt as the product of an asexual generation. Other individuals of the same species give rise to broods of anisospores which are interpreted as microgametes and macrogametes representing the sexual generation.

In Mycetozoa multiple division is characteristic but complicated by the typical plasmodium nature of the organisms. Such plasmodia are formed usually by the plastogamic union of amœbæ arising from spores, the nuclei remaining separate and thus forming a multinucleated protoplasmic aggregate. Many of these nuclei degenerate (Kränzlin, Jahn); some become active agents in the formation of specialized structures of the fruiting bodies (elaters, etc., Kränzlin, 1907); others divide by mitosis to form nuclei of the spores contained with the elaters in the spaces of a meshwork formed by a special protective and supporting part of the fruiting bodies called the capillitium (Fig. 146, p. 328, see also p. 326).

Multiple division in the Sporozoa is characteristic of practically all Coccidiomorpha, particularly in agamogony. The nuclei divide repeatedly by mitosis until many are formed, after which the body plasm breaks up into as many agametes as there are nuclei. In

many cases a portion of the old cells is left unused or not included in the protoplasm of the offspring. Thus in *Plasmodium vivax* and other malaria organisms, the pigmented granules (melanin) are left behind when the agametes separate (Fig. 120); in many coccidia the agametes are oriented in respect to such residual products. Multiple division is also characteristic of the developing zygotes of gregarines and hæmamœbidæ, the eight sporozoites of gregarines and the multitude of sporozoites of *Plasmodium* being formed in this manner.

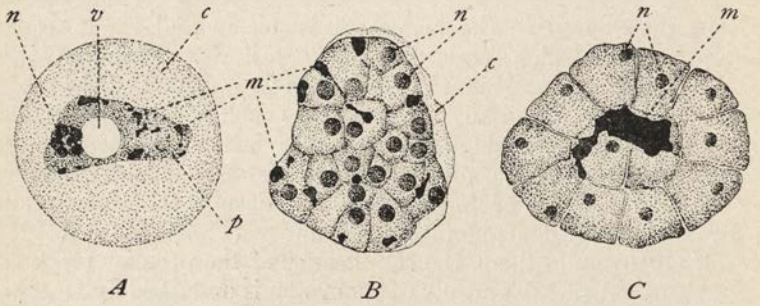

FIG. 120.—Malaria organisms. *A*, *Plasmodium vivax* in blood corpuscle; *B*, same in agamete formation with distributed melanin (*m*). *C*, *Plasmodium malariæ*, agamete formation with concentrated melanin. *c*, red blood corpuscle; *m*, melanin; *n*, nuclei; *p*, parasite; *v*, vacuole. (After Calkins.)

In the above account of the reproductive activities of the Protozoa no attempt has been made to give an exhaustive treatment, but other examples will be given in the following chapters on classification.

In many cases in the above description there is evidence of reorganization of the protoplasm and evidence that may be interpreted as supporting Child's view of de-differentiation as an offset to the accumulation of products of metabolism which hamper further metabolic activities (p. 208). Some of this evidence is given in connection with the phenomena of equal division, particularly in division of the ciliated forms and the conclusions reached are in agreement with Child's. Hartmann, also, comes to a similar conclusion in connection with the cultural history of *Eudorina elegans* (1923) and from merotomy experiments with *Amœba polypodia* (1924). In the latter an individual was cut in two fragments; the nucleated part regenerated but instead of permitting it to divide it was cut again when fully grown. This process was repeated until the original amœba had been cut 32 times in forty-two days and without an intervening division. The control amœbæ from the same clone divided 15 times in the same period. This experiment would appear to confirm Child's argument that amputation of a part of the differentiated protoplasm would effect a partial rejuvenescence, and Hartmann interprets it in this way: "Repro-

duction," he says, "may rightly be interpreted as a process of rejuvenation. Our continued amputations in these experiments provide a substitute for the rejuvenating effect of reproduction" (1924, p. 458). His further conclusion that his results "indicate experimentally, a potential immortality of the protozoan individual" (p. 456) can scarcely be allowed on the basis of forty-two days' experience. A single individual of *Uroleptus mobilis* has lived for more than ninety days without dividing, and similar but younger individuals have been cut as in Hartmann's experiments, to find out if ciliates would sustain Child's conclusion. The results (not published) were invariably negative, although *Uroleptus* is an excellent type for this kind of work and invariably undergoes rejuvenescence after conjugation and after endomixis (see Chapter XII).

With unequal division by budding and multiple division there is further evidence of reorganization with reproduction. The small cells that are budded off contain none of the differentiated cellular elements of the parent organism. The spores are likewise provided with protoplasm whose activities are unhampered by accumulated products. This is clearly evident in the asexual reproduction of *Plasmodium vivax* (p. 242), and is well illustrated in forms where specialized structural elements are indications of the differentiations which the old protoplasm has undergone. Thus in Mycetozoa some of the hundreds of nuclei degenerate and give rise to spiral elaters which with their spiral walls are made up of microsomes and kinetic elements (Strasburger, Kränzlin), while parts of the protoplasm become differentiated into encrusting peridia and supporting capillitia. All of these differentiations are left behind when the spores are formed and distributed. Analogous somatic structures are also characteristic of the spore-forming stages of some types of Gregarinida and Myxosporidia. In the former the spore-containing organs are either relatively simple spore cysts as in *Monocystis* types (Fig. 179, p. 420) or more complicated structures—sporangia—of some polycystid gregarines (*e. g., Echinomera hispida* or *Gregarina cuneata*). In the former the spores are dispersed by the formation of gas which bursts the cyst membranes. In the latter, finger-formed tubes are developed from the peripheral protoplasm of the cyst. These are formed from residual "chromidia" which collect in rings about the periphery and from which the finger-formed tubes grow into the mass of developing zygotes (Fig. 121).

When the cysts are mature absorption of water causes the rupture of the cyst walls, the tubes are forced out and evaginated as an inturned glove finger may be blown out. The spores then are distributed through these hollow tubes or sporoducts.

In Myxosporidia still more complicated structures recalling the capillitia of Mycetozoa, are characteristic of the spore-forming stages. In *Sphæromyxa sabrazesi* according to Schröder (1907) and in *Myxobolus pfeifferi* according to Keysselitz (1908) the internal

bud (pansoproblast) which is destined to form the spores, contains two nuclei, one of which is smaller than the other. These nuclei increase by division until there are 14 altogether; 2 of these degenerate without further function, and the remaining 12 are divided into two groups of 6 each, the protoplasm dividing with them to form two protoplasmic multinucleated bodies which will develop into sporoblasts (Fig. 186, p. 447). Of the 6 nuclei in each cell, 2 are "somatic" and take part in the formation of the shell or capsule of the sporoblast; 2 others are also "somatic" and participate in the formation of the polar capsules and threads characteristic of the Cnidosporidia; the remaining 2 nuclei persist as germinal

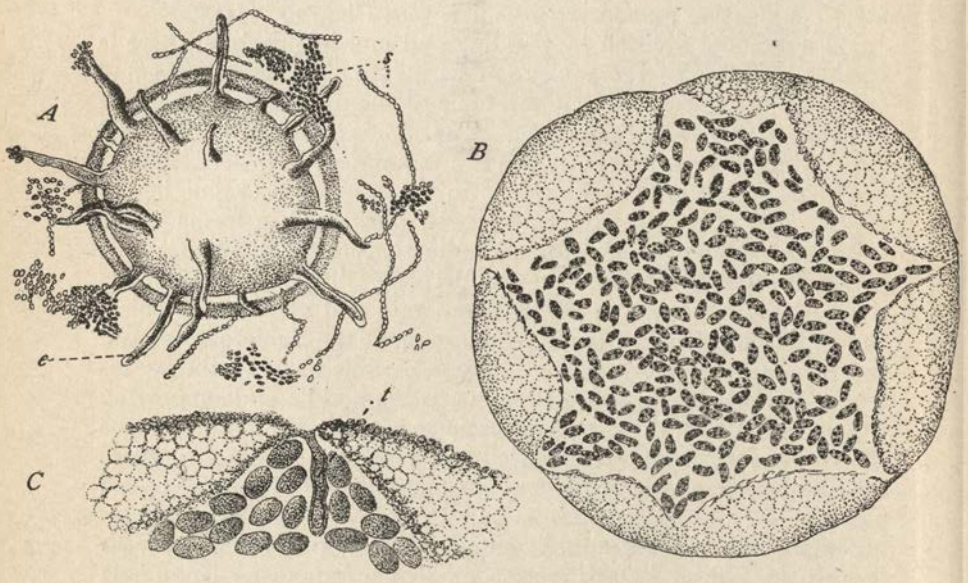

FIG. 121.—*Gregarina cuneata*. *A*, surface view of sporocyst with ripe sporoblasts issuing from sporoducts (*e*). *B*, *C*, sections of sporocyst with ripening spores and developing sporoduct (*t*). (From Calkins after Kuschakewitsch.)

nuclei which, according to observations of several different authorities, later fuse into one (p. 547).

In all of these cases the specialized structures accompanying spore formation are formed only at one period in the life cycle and a period which comes at the end of long-continued metabolic activity. They represent therefore, a differentiated protoplasm which is not evident in the protoplasmic make-up of the progeny. What is true of these visible differentiations is also probably true of analogous differentiations which are not visible, and we have reason to believe that the products of unequal division and of multiple division are not encumbered by protoplasmic conditions which hamper vitality—in other words, that they have undergone rejuven-

escence. Such young forms have again the potential of vitality of the genotype and are able to go through the series of differentiations which are characteristic of the life of the genotype.

IV. DEVELOPMENT.

In Metazoa, development starts with the fertilized egg and consists in the progressive formation of organs and organ systems by differentiations, and grouping of differentiated cells. A strict comparison of Protozoa with Metazoa in development would involve the history of a fertilized cell through all phases of asexual reproduction (comparable with somatic cell division) to the gamont stage. Only by a fanciful interpretation, however, can the entire progeny of a single fertilized cell of Protozoa be regarded as an individual similar to a metazoön, although there are similar phases of vitality which may be indicated in common by the terms youth, maturity and age (see Chapter X). The protozoan "individual," however, is a single cell and as usually seen is in the agamont stage. In the majority of Protozoa little or no development is necessary, the daughter cells being almost perfect individuals when formed and similar enough to the parent to be mistaken for nothing else. Here the only processes that can be regarded as development are those which have to do with the formation of shell structures, as in Dinoflagellata, *Coleps hirtus*, etc., and the new development of anterior parts of posterior daughter cells and posterior parts of anterior cells.

It is quite different, however, with the products of multiple budding or of multiple division. Here the young forms are unlike the parent and during growth, undergo changes which may properly fall under the heading of development. In some cases, for example in Foraminifera, Mycetozoa, and Sporozoa, the small fragments produced by a parent require fertilization in order to develop. The zygote of *Polystomella crispa* or of *Trichosphærium sieboldi*, formed by the fusion of flagellated gametes (flagellispores) develops into the asexual generation by protoplasmic growth and nuclear division, but without cell division, development of the former being indicated externally by the formation of a many-chambered shell. Similarly in the Mycetozoa the zygote formed by amœboid or flagellated gametes develops into a plasmodium by cell fusions and nuclear divisions.

In the Sporozoa the zygotes, formed by union of similar gametes (isogametes) or of dissimilar gametes (anisogametes) undergo a variable number of metagamic divisions, three in the majority of Gregarinida and two or more in the Coccidiomorpha. The end-result of such metagamic divisions is the formation of two or more similar sporozoites which are entirely different from the adult individuals and undergo a more or less complex development. When they are introduced into a new host the sporozoites are liberated

from their capsules, or introduced naked into the blood by some intermediate host. They make their way to the definitive site of parasitism, penetrate into cells and begin their development. In the simpler gregarines only the young stages are passed in such host cells and growth is not accompanied by any marked structural differentiations. In the polycystid gregarines the parasite never becomes entirely detached from its host cell until it is fully mature and de-differentiation begun by the loss of the attaching organ (epimerite). With its growth the body becomes differentiated into an anterior chamber (protomerite) and a nucleus-holding posterior chamber (deutomerite) and in the different species these three portions of the cell become variously ornamented and specialized. The epimerite particularly becomes modified in different ways that are useful for purposes of anchorage. It may be a mere ball of

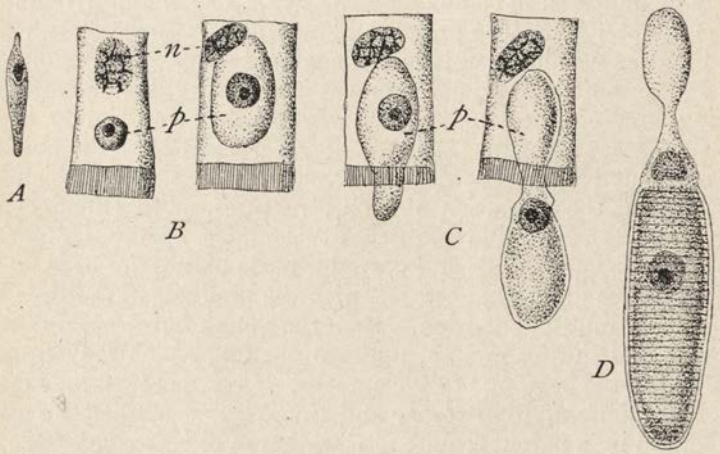

FIG. 122.— Development of a polycystid gregarine (schematic). (After Wasielewsky.)

protoplasm as in *Gregarina longa;* a spade-shaped structure as in *Pileocephalus herri;* a long knobbed proboscis either simple or provided with spines as in *Stylorhynchus longicollis* or *Geniorhynchus monnieri;* or there may be many finger-form processes as in *Echinomera hispida* or thread-like processes as in *Pterocephalus giardi.* In *Corycella armata* it becomes a single crown of hooks; in *Beloides firmus* hooks combined with a lone spine. While these epimerites serve primarily for attachment, they also serve, in some cases at least, as food-getting organs which they take at the expense of the host. In *Pyxinia mœbiuszi* the epimerite forms a long haustoria-like process which extends through the epithelial cell of the gut and into the blood lacunæ of the submucosa (Fig. 93, p. 196) and in *Stylorhynchus longicollis* a canal is said to extend from the tip of the epimerite through the primite and into the deutomerite of the

parasite serving for the passage of food (Leger). An extreme case of gregarine differentiation has been described by Drzewiecki in *Stomatophora coronata* in which mouth, peristome and anus are said to occur. (Reference from Doflein, 1916.)

The buds of Suctoria have a rather complicated developmental history, especially in forms whose "embryos" are parasitic in other Protozoa (*Sphærophrya* species). The buds possess cilia which are arranged in different patterns in the various species, and by which they swim actively about until they finally settle down for development. They also possess, as a rule, some longer cilia at the anterior end which have been homologized with the adoral zone of the ciliated Infusoria, and at the posterior end they possess a sucking disc by means of which the buds attach themselves to some solid object either living or lifeless, and from which a stalk is developed. With growth of the stalk the cilia are absorbed and tentacles—suctorial, piercing or seizing—are developed. In the parasitic forms the ciliated embryos may develop tentacles while in the motile condition, but on coming in contact with a quondam host, cilia and tentacles are absorbed and as an ectoparasite the young form makes a pit in the cortex of the host. It may then reproduce by cell division in this pit until as many as 50 or more are produced, and these escape through a slit-like birth opening of the improvised brood pouch.

In some types of Protozoa finally, especially in the colonial flagellated forms, the single cell undergoes a series of cleavage stages the sequence of which is similar to that of many types of eggs of Metazoa. This is particularly striking in forms like *Gonium pectorale, Platydorina caudata, Stephanosphæra pluvialis,* etc., which, as adults, consist of definite numbers of cells arranged in definite patterns (Fig. 3, p. 21).

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CHAPTER VI.

SPECIAL MORPHOLOGY AND TAXONOMY OF THE MASTIGOPHORA.

The Protozoa are usually grouped as a phylum of the animal kingdom, notwithstanding the fact that many of them are much more plant-like than like animals. They have also been regarded, quite generally, as the "lowest" types of animals from which the Metazoa have been evolved, various classical theories by Haeckel, Lankester, Bütschli and Metschnikoff attempting to trace back the metazoön gastrula to prototypes amongst the colonial flagellates many of which are much more closely related to the higher plants than to Metazoa (*e. g., Volvox, Synura, Gonium*, etc.). It is quite conceivable, as Franz (1919) has pointed out, that such theorists have started with a fundamentally wrong assumption and that all Protozoa, instead of being primitive, have been derived from higher plants or animals. The latter point of view, which falls in line with the startling suggestion elaborated in Bateson's presidential address of 1914 has much to recommend it, although much might be said against it. It is useful at any rate, if only to challenge the easy assurance so evident in most general zoölogical text-books, that Protozoa are primitive animals and that Metazoa have been derived in direct line from them.

Protozoa, primarily, are single-celled organisms which, together with bacteria and the single-celled plants, comprise the group to which Haeckel's term Protista (1868) has been applied. Protista, or even Protozoa, as Newman (1924) has suggested, may well be regarded as a separate kingdom of living things with many characteristic features of the plant kingdom on the one side, and of the animal kingdom on the other. It includes the phylum Bacteria, many of the Protophyta, and the phylum Protozoa, each with indefinite boundaries. Of these the Protozoa alone are essentially animal-like, but, through the chlorophyll-bearing forms they interdigitate closely with the Protophyta, and through the Spirochætida with the Bacteria.

CLASSIFICATION OF THE PHYLUM PROTOZOA.

The inadequacy of any formal statement to convey an idea of the range of forms, structures and activities of the Protozoa is recog-

nized by all who are familiar with the group. A glimpse of the variety may be possible in a brief summary of the classification which is based in the main upon general organization for carrying out the fundamental activities. The four great groups—Mastigophora, Sarcodina, Infusoria and Sporozoa, are natural groups with the exception of the Sporozoa: The first characterized by motile organs when present in the form of vibratile *flagella;* the second by protoplasmic

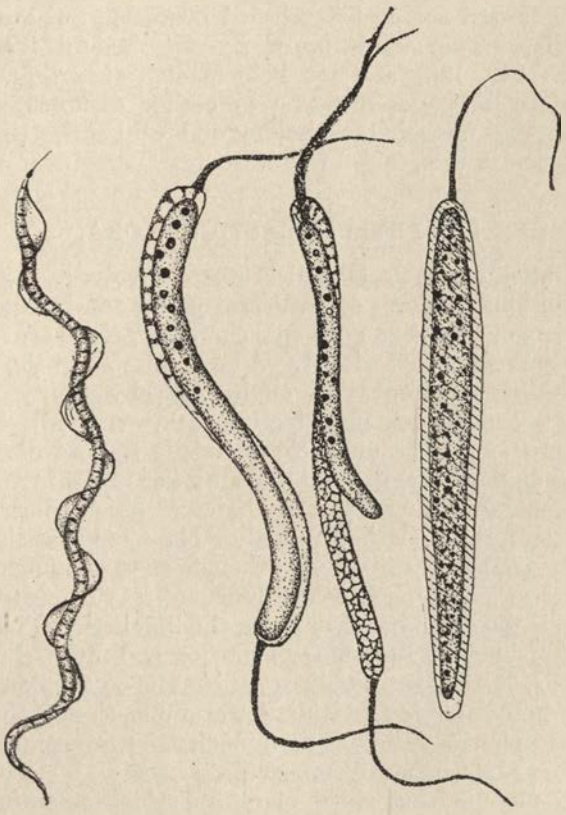

FIG. 123.—*Cristispira anodontæ* with spirally wound crista; and flagellum insertion in bacteria. (Former from Fantham, latter from Bütschli.)

projections known as *pseudopodia,* some types of which are motile organs. In the third group motile organs are in the form of minute lash-like *cilia,* which are invariably present in some stage of the life cycle. The Sporozoa finally are characterized by the general absence of motile organs, by the invariably parasitic mode of life, and by the method of reproduction.

The four main groups of Protozoa should have the taxonomic

value of sub-phyla, each with its own peculiar type of structural and functional differentiations. Of these we regard the Mastigophora as the central group from which the present day Sarcodina, Infusoria and Sporozoa have been derived, either directly or indirectly, evidence of which has been given in the preceding chapters.

The Spirochætida are not included here, as their main characteristics place them much closer to the bacteria than to Protozoa. Their transverse division and spore formation through coccoid bodies are not duplicated amongst flagellated Protozoa, but are distinctly Spirillum-like. The columella of *Spirochæta* and the crista of Cristispira (Fig. 123) are not homologous with the undulating membrane of Trypanosoma as was earlier assumed. In short, there is nothing in their morphology or life histories that justifies their inclusion in the group of Protozoa.

SUB-PHYLUM MASTIGOPHORA.

Classification of the flagellated Protozoa involves an old problem of distinguishing between animals and plants, and while the difficulty is more or less of an academic character, there are some practical problems connected with it. A large number of the flagellated Protozoa possess chlorophyll through which, by energy of the sunlight, they are enabled to manufacture their own food. Such autotrophic nutrition is the most characteristic feature distinguishing plants and animals, but the real difficulty lies in the fact that many such chlorophyll-bearing forms are heterotrophic in nutrition. To classify such forms as either animal or plant is unsatisfactory and it seems best to frankly admit that they lie on the boundary between the two great groups. Another difficulty in classification of the flagellates is the difficulty of drawing the line between bacteria and Protozoa, or between unicellular Protozoa and multicellular plants and animals. Here again, certain organisms lie on the boundary line. *Volvox* and other phytomastigida, for example, closely approach the many-celled plants. There is no difficulty, however, in distinguishing between Metazoa and Protozoa.

The sub-phylum, as a whole, manifests a high potential of evolution with highly developed powers of adaptation, resulting in the most diverse modes of life from intracellular parasitism to life in the purest drinking water. The typical form is elongate and ovoidal, variable, however, in many cases of pseudopodia-bearing forms, or variable by virtue of the highly metabolic plasm. The cortex, however, in many cases may be firm and rigid, resulting in a permanent form of the cell. Highly sculptured membranes of pseudochitin or cellulose are frequently present; gelatinous secretions are prevalent. These may be of pseudochitin or cellulose and frequently form a matrix in which the individual cells lie.

Colonies thus arise of gregaloid, sphæroid, arboroid or catenoid nature. In many of the plant-like forms, the motile stage is frequently transitory, the cells throwing off or withdrawing their flagella, secreting jelly and passing into a resting stage. Here, they may reproduce by division, giving rise to aggregates known as the *Palmella* phase. In some cases, this phase is dominant, the motile stage being extremely short; in other cases, the motile stage is dominant and the Palmella-stage short. This condition must be distinguished from encystment where the cells secrete a resistant membrane within which they are able to withstand adverse external conditions.

A typical flagellum consists of a periplastic sheath continuous with the periplast of the cell, and an axial, highly contractile filament. The latter penetrates more or less deeply into the endoplasm where it arises from the substance of a definite kinetic center. The number of flagella is highly variable, sometimes 1, 2, 3, 4, 6, 8 and many being characteristic of different species. When more than one are present, they may be similar in size and structure or diverse. In the latter case there may be one main locomotor flagellum and a second more minute accessory flagellum. In some cases, one flagellum is directed anteriorly, the other posteriorly. The latter is usually larger, heavier, and less active, behaving like a trailing flagellum or a runner on which the cell moves over the surface. It is possible that such a trailing flagellum may remain or become attached to the body periplast which may be drawn out ledge-like, and cause it to vibrate or undulate with the movements of the flagellum. Such may be the possible origin, in some cases at least, of the undulating membrane found in a number of types. The insertion of the flagellum varies. In some cases a simple endoplasmic basal granule or blepharoplast is the sole representative of a kinetic complex. In other cases the basal body, giving rise to the flagellum, is separated from the blepharoplast, the two kinetic centers being connected by a rhizoplast. In other cases all kinetic bodies may be concentrated in one relatively large kinetic center; or the parabasal body, basal body and blepharoplast may be separate elements in the cell connected or not by rhizoplasts. This matter of insertion is one of the essential differences between the so-called flagella of bacteria or spirochætes and animal flagellates. In the former the axial filament is absent, the so-called flagellum arising from the periplast (see Chapter II).

Pseudopodia, in addition to flagella, are common throughout the entire group, and as in Sarcodina, may be of the axopodia or lobopodia type. In forms with axopodia the close resemblance between the flagella and the pseudopodia in respect to origin and motility lends support to the view that the two are homologous structures and that axial filaments of heliozoön pseudopodia and axial fila-

ments of flagella belong to the same category of kinetic elements (p. 141). Filopodia or ray-like pseudopodia without axial filaments, are present in some of the Chrysomonadida; lobopodia are widely distributed in the same group and in the Rhizomastigidæ.

A definite cytopharynx even in the chlorophyll-bearing forms is frequently present, in which case the flagella arise from its walls. The function in many cases is only conjectural, but in other cases it acts as a cell mouth or cytostome for ingestion of solid food, and in still other cases provides a receptive area for intake of food in saprozoic forms. Contractile vacuoles usually empty into it either directly or through the medium of reservoirs and canals.

Contractile vacuoles are either simple or complex. The simple vacuoles have no accessory structures; the complex vacuoles form a vacuole system consisting of smaller contractile vesicles which empty their contents into a common reservoir, the latter being connected with the outside by a longer or shorter canal. Such complex systems are characteristic of the Euglenida and the Chloromonadida. Non-pulsating vesicles known as pusules, serving a hydrostatic function, are characteristic of the Dinoflagellida.

Nuclei are either vesicular or massive in type, the former predominating. A single nucleus is the rule but there may be two (*Giardia*) or many (*Calonympha, Sphæronympha,* etc.). A central nuclear mass (endosome) is typical and may be a combination of chromatin, plastin, or chromatin and plastin, with a kinetic (endobasal) body. The division figure assumed by the nucleus during reproduction may be one of an enormous number of variations.

The classification of flagellated Protozoa is not yet on a satisfactory basis and a "natural" system appears to be impossible with our present limited knowledge. Any classification, however, should be considered a means to an end and, except for a few specialists, not an end in itself, and any system which furnishes a comprehensive idea of the wealth of form and function in these Protozoa is adequate until a better one is forthcoming. Bütschli's (1884) system based upon the nature and number of the flagella was superseded by that of Klebs (1893) who found that the nature of the anterior end or mouth-bearing parts gives a better means of grouping than do the variable flagella, especially when combined with the method of nutrition. Senn (1900) adopted the same principle for his major groups while the larger sub-groups were based upon the nature of the contractile vacuole and the vacuole system. In his classification the nature of the cortex and its secretions in the form of gelatinous membranes, tests, shells, stalks, etc., formed the basis of generic distinctions, together with the number and structure of the flagella, metaboly or rigidity, undulating membranes, etc. This system was adopted on the whole, by the majority of protozoologists including Minchin (1912) and Doflein (3d edition) until

it was superseded by the more natural classification of Pascher and Lemmermann (1912) which, in the main, is adopted here.

The chlorophyll-bearing forms are fairly homogenous and together with the colorless forms which by reason of their structures and life histories belong with them, form a large division of the Mastigophora. Other colorless forms, not obviously related to chlorophyll-bearing types, form a second large division. These two divisions are given the value of classes in the present work and with their subdivisions will be considered in accordance with the following classification:

Sub-Phylum Mastigophora, Diesing.
- Class I. Phytomastigoda (Phytomastigina of Doflein)
  - Order I. Chrysomonadida
  - Order II. Cryptomonadida
  - Order III. Dinoflagellida
  - Order IV. Phytomonadida
  - Order V. Euglenida
  - Order VI. Chloromonadida
- Class II. Zoömastigoda (Zoömastigina, Doflein)
  - Order VII. Pantastomatida
  - Order VIII. Protomastigida
  - Order IX. Polymastigida
  - Order X. Hypermastigida

Class I. PHYTOMASTIGODA. Doflein em.

In this group of Mastigophora are included not only the flagellated forms which by virtue of their chlorophyll are able to live in a typical autotrophic manner, but also those forms which, although they are colorless, nevertheless by their structures and life histories show unmistakable relationship to the chlorophyll-bearing flagellates. It cannot be claimed that the arrangement of orders within the class represents an ascending scale in complexity of structures or functions. Each order includes some forms which are relatively simple, others complex, in regard to nuclear and kinetic structures, vacuole system, and cell membrane. In two orders only, the Euglenida and the Chloromonadida, are the genera all of a higher type of organization, while the Phytomonadida, with obligatory autotrophic nutrition, are much more advanced in respect to sex differentiations than are the representatives of other orders.

Many of the phytomastigote flagellates are extremely small (2 to 4 μ), but the great majority measure from 25 to 100 μ in length, while some exceptional types are relatively large, *Noctiluca miliaris*, for example, with a diameter of 1 to 1.5 mm.

The typical form assumed is monaxial and ellipsoidal, but there are wide variations. Many are polymorphic, passing from sym-

metrical to asymmetrical stages and back again; many of these are amœboid, especially in the Chrysomonadida and Cryptomonadida; others, particularly the Euglenida, are sufficiently plastic to undergo form changes without break or rupture of the periphery as happens in pseudopodia formation. Such plastic organisms are said to be metabolic and the phenomenon is usually called metaboly.[1] Pseudopodia formation is found in many different types of flagellates and conversely in some rhizopods flagella are present at some stage of the life history. Whether to classify such forms as flagellates or rhizopods is largely an academic question which may be answered in an arbitrary way by including all colored forms with pseudopodia amongst the Phytomastigoda provided a flagellum is present at any stage, while in some sub-orders (Rhizochrysidina, Phytodinidæ, etc.) even flagella are unknown, the yellow, chromatophore-bearing stages alone having been described.

Amœboid and Metabolic Types.—In all cases of amœboid and metabolic forms the cell symmetry is variable by reason of the form changes due to transformation of energy as a result of destructive metabolism, in the absence of firm and resistant membranes, pellicle, or products of the cortex in the form of lifeless membranes, shells, etc. When such cortical structures are present the cell form is usually constant, although metaboly may still be observed (Euglenida). The cell membrane may be delicate or heavy; plain or striated; smooth or ridged or spinous, and the outline may be simple or spirally twisted. It may be covered by gelatinous secretions or impervious cellulose membranes (Dinoflagellida, Phytomonadida, Chloromonadida) or by calcified shells (*Phacotus*) or plates (*Coccolithophorida*) or by silicious plates (*Mallomonas*) or skeleton (*Distephanus*). In some cases the pseudopodia that are formed are tentacle-like and are regularly arranged about the base of the flagellum (*Cyrtophora*, *Pedinella*, etc.).

Flagella.—Flagella vary in number from 1 to 4, very exceptionally more than 4. If 2 are present they may be similar in length and in function (isomastigote) in which case both are primary flagella; or they may be dissimilar in form and size (heteromastigote) and directed forward, in which case the larger is the primary, the smaller the secondary, or there may be 2 or 3 secondary flagella. In many cases one (primary) flagellum is directed forward, while a second which may be either larger or smaller than the primary, is directed backward, and is known as a trailing flagellum (Schleppgeissel). In Dinoflagellida one flagellum moves freely in the surrounding water while a second undulates in a characteristic transverse groove

[1] The use of the term *metaboly* in this connection is misleading, metabolism meaning the sum-total of chemical processes in the upbuilding and breakdown of living protoplasm; all living things are metabolic in this sense and in using such a term with a double meaning the significance is carried with the connotation.

or girdle around the cell, giving the impression of an annular row of cilia which led to the earlier name Cilioflagellata for the group (Fig. 70, p. 136). Multiple flagella are characteristic of the Polymastigida and Hypermastigida.

Nuclei for the most part are simple vesicular and endosome-bearing nuclei, but there are wide variations in complexity so that a general description is quite inadequate (see p. 56).

Chromatophores and Stigmata.—Chromatophores are the most characteristic of the plastids in this group. While usually present as definite bodies of characteristic size and shape, they are sometimes in the form of a vague network (Fig. 124), or as irregular clumps of chlorophyll-holding substances (Chrysomonadida, Cryptomonadida). More often, however, they are definitely formed bodies, discoidal, cup-shape, band-, star-, or rod-form. Their colors vary from yellow

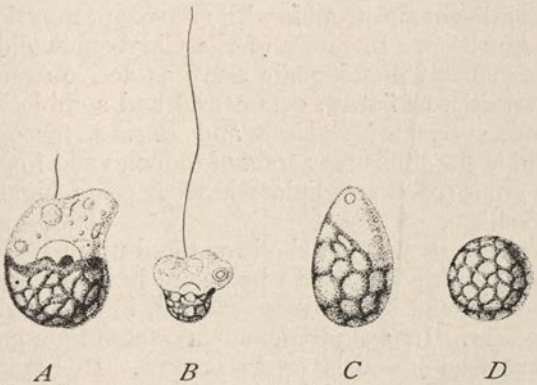

FIG. 124.—Flagellates with chlorophyll in a reticulated network. *A*, *B*, *Chrysapsis sagene; C*, *D*, *Chrysapsis fenestrata.* (After Pascher).

and brown (Chrysomonadida, Cryptomonadida, Dinoflagellida) to a bright green in the Phytomonadida, while blue-green and red are occasionally seen. In all cases the basic color is chlorophyll-green, varying in shade as it does in the higher plants; this is often masked by overlying colors which in all cases are readily dissolved out by alcohol, thus exposing the typical green.

Many of the chlorophyll-bearing flagellates and a few colorless ones (Astasiidæ) possess a minute rod-shape, oval or discoidal mass of red pigmented oily (lipochrome) substance called the stigma or "eye-spot," which is usually situated in the anterior end. According to Françé, it may be accompanied by one or more paramylum bodies which function as a lens system. Following Engelmann the stigma is generally interpreted as a bit of protoplasm particularly sensitive to light rays (see Mast, 1916, 1923). Observations are conflicting

as to its reproduction by division in different forms. In *Euglena* according to Zumstein (1900) it divides longitudinally, whereas in *Uroglena* according to Iwanoff (1899) it is newly formed at each division of the cell.

Trichocysts.—Trichocysts giving rise to gelatinous threads on irritation and possibly functioning as adhering organoids are found in the cortex of some of the Chloromonadida. Much more complex cnidocysts are present in some of the Dinoflagellida (*Pouchetia*, *Polykrikos*).

Nutrition.—Nutrition in Phytomastigoda, as indicated by the presence of chromatophores and chlorophyll, is essentially autotrophic. Only a few types, however, are constrained by reason of an unbroken cellulose covering to live exclusively by autotrophic means (Phytomonadida). In all other groups more or less well-defined areas for absorption of fluid or solid substances are present and we find a combination of autotrophic with either saprophytic, saprozoic, or holozoic nutrition. In many of the Chrysomonadida the temporary or permanent pseudopodia serve as active agents in food-getting. Parasitic, or better, commensal and symbiotic types are not unknown, species of *Euglena* and *Phacus*, for example, are usually found in the alimentary tract of tadpoles and frogs (Hegner). The entire group of Blastodinidæ have become highly modified through parasitism.

Products of destructive metabolism stored up in the cell are frequently characteristic enough to be useful in classification. Thus starch is never found in Chrysomonadida, but leucosin, oils and fats are present. In Cryptomonadida starch-like products are abundant but true starch is rarely present. In Euglenida paramylum is characteristic and in the Phytomonadida true starch. In many cases, but not of necessity, the carbohydrates are manufactured in connection with definite bodies, the pyrenoids.

While the Phytomastigoda are typically motile organisms, moving by means of flagella, the majority of them differ from other motile Protozoa in having the ability to discard their flagella and still continue an active metabolic life. Such phases are not to be confused with encystment, which involves impervious walls, but while in this immobile state feeding and reproduction may continue normally. The Rhizochrysidæ and Phytodinidæ include forms in which flagella have never been seen and apparently represent forms in which such, usually temporary stages of other types, have become permanent. Not infrequently and while in this aflagellate stage, the cells secrete a gelatinous enveloping substance and in this, with reproduction, typical Palmella-like aggregates are formed. Such quiescent phases are common in all orders of the group with the exception of the Chloromonadida and become the dominant phase in the life history of the Chrysocapsinæ, Phæocapsinæ, Phytodinidæ, and

Tetrasporidæ. Such Palmella-stages are difficult to distinguish from the lower algæ.

Reproduction is invariably by division, usually and typically by longitudinal division, exceptional transverse division occurring in relatively few forms (*Oxyrrhis*, *Polytoma*, *Parapolytoma*, etc.). In most cases there is a doubling of the characteristic cell organs—nucleus, blepharoplast, parabasal body, flagella, chromatophores, etc., although in some authentic cases the flagella and basal bodies are discarded and new ones are formed (see p. 210). In many cases, also, division does not occur during the motile phase, but only after the flagella are thrown off and the individual goes into a resting phase analogous to the Palmella-stage. Such quiescent individuals form what are called "division cysts" within which reproduction occurs (*Euglena spirogyra*, *Euglena gracilis*, etc.).

Sexual processes are well developed in the Phytomonadida, very questionable in Euglenida, and have never been observed in Chrysomonadida and Cryptomonadida.

Permanent cysts with resistant walls are known in relatively few forms, and where found they seem to be simpler the more complex the organism and *vice versa*. The cysts usually contain an excess of reserve foodstuff in the form of oil, starch, paramylum, etc.

Most of the Phytomastigoda have the ability to secrete gelatinous substances from the cortex through the pellicle and so to form a gelatinous matrix in which the cell lies. Upon reproduction many cells are thus enclosed in jelly with, usually, the flagella extending through it to the outside. In this way spheroidal colonies are formed in many cases (*Syncrypta*, *Uroglena*, *Uroglenopsis*, *Chrysosphærella*, etc.). The secretion of substance (cellulose, gelatinous, chitinous) from the posterior end of the cell results in the formation of definite stalks. These may or may not be accompanied by tests or houses which may fit the cell tightly, in which case the test is secreted from the entire periphery of the cell (*Chrysococcus*, *Trachelomonas*, *Dinoflagellata*), or it may be considerably larger than the contained cell, in which case it is first secreted from the cell as a whole and later from only a distal expanded portion as in *Dinobryon*, *Hyalobryon*, etc. (Senn).

The majority of Phytomastigoda are widely distributed in both salt and fresh water. The Coccolithophoridæ, Silicoflagellidæ and the majority of the Dinoflagellida, are exclusively marine. The Chlamydomonadidæ and most of the Volvocidæ on the other hand are limited to fresh water. Many of them are decidedly planktonic and clear lakes and water supplies often contain them in such numbers as to impart distinct colors (red, yellow, green), odors and tastes (especially *Synura* and *Uroglenopsis*) to the water. Iron in the water leads to abundant growth of *Trachelomonas*; nitrogen, to various types of Euglenida and Cryptomonadida. Many of them

are symbiotic as in the yellow cells of Radiolaria and Foraminifera (Zoöxanthellæ especially *Chrysidella*). Some are also ectoparasitic on diatoms, filamentous algæ and Crustacea; others are endoparasitic in water-dwelling animals such as Gammarus and allied Crustacea, and on rotifers.

Order I. CHRYSOMONADIDA.

The Chrysomonads are characterized by the presence of yellow-brown chromatophores; by the presence of oils and highly refractile granules (leucosin); by spore formation in cysts and by the silicious composition of the cyst walls. Starch is absent. The chemical nature of the coloring matters is not yet satisfactorily made out. For *Chromulina rosanoffii*, Pascher (1913) states that chrysochlorophyll, phytochrysin, and chrysoxanthophyll have been identified although whether or not identical with similar chromophyll substances in higher plants is unknown.

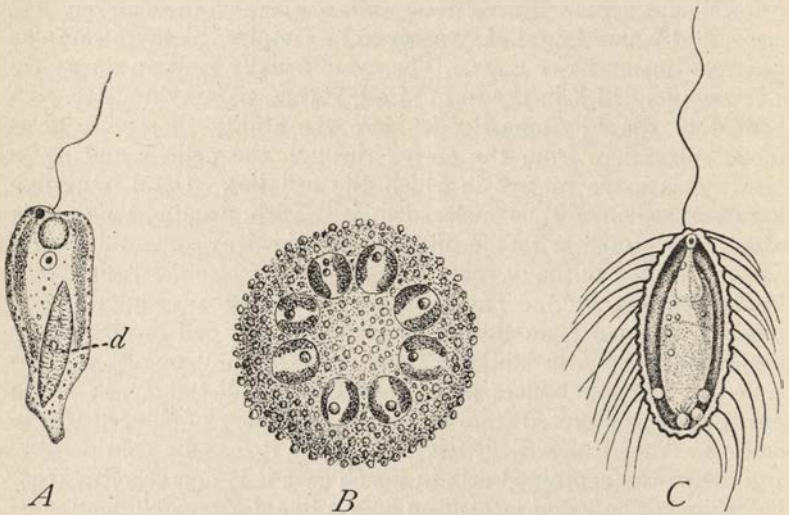

Fig. 125.—Types of Chrysomonadida. *A*, *B*, motile and Palmella stages of *Chromulina flavicans*. (From Calkins after Bütschli.) *C*, *Mallomonas plœsslii*. (From Doflein after Klebs.)

The flagella are always inserted apically and are one or two in number, the number and relative sizes determining some of the families. The monads are comparatively simple in structure and the body is usually regular and without dorso-ventral differentiation. The pellicle is usually delicate and inconspicuous but may be heavy and provided with keels, ridges, flanges, etc., or covered by fine silicious plates as in *Mallomonas* (Fig. 125). Cups and houses of cellulose, often colored by iron oxide, are abundant and frequently

of exquisite design (*Dinobryon, Derepyxis, Chrysopyxis* (see Fig. 126, etc.).

Nutrition is primarily autotrophic but there is frequently a combination of both autotrophic and holozoic or saprozoic methods. The

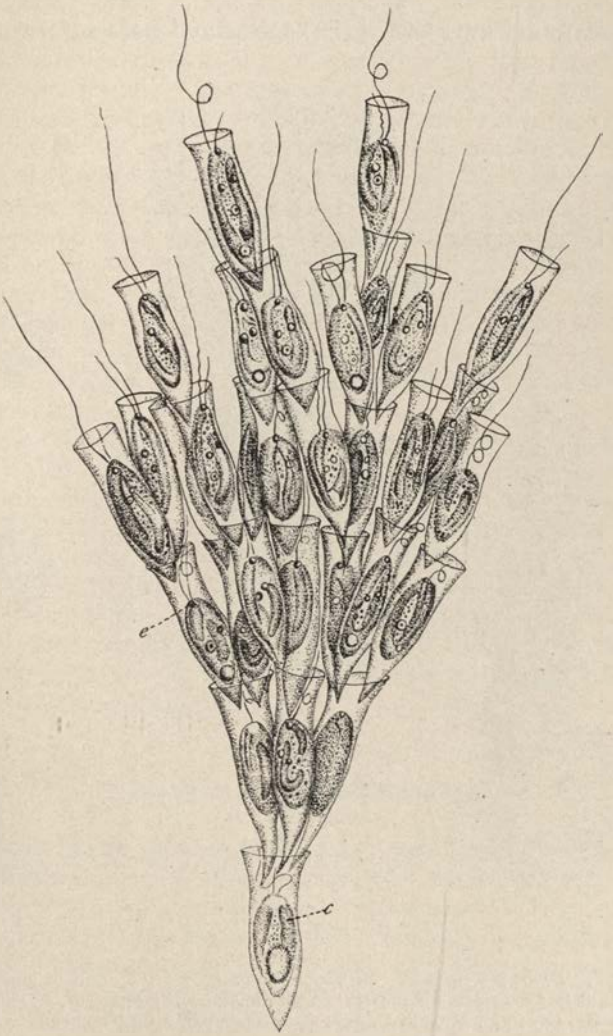

FIG. 126.—*Dinobryon sertularia.* (From Calkins after Stein.)

entire body is frequently amœboid and pseudopodia serve for food taking. In *Cyrtophora, Pedinella* and *Palatinella* the pseudopodia are arranged like tentacles of a sea anemone and function in much the same manner (Fig. 94, p. 200).

Conjugation and fertilization are entirely unknown in the Order and reproduction is mainly by longitudinal division which may lead to catenoid (*Chlorodesmus, Cyclonexis*), arboroid (*Dinobryon, Hyalobryon*, Fig. 126) or spheroidal (*Synura, Uroglena, Uroglenopsis, Chrysosphœrella, Syncrypta*) colonies. Many of the Chrysomonadida round out, lose their flagella and pass into a quiescent

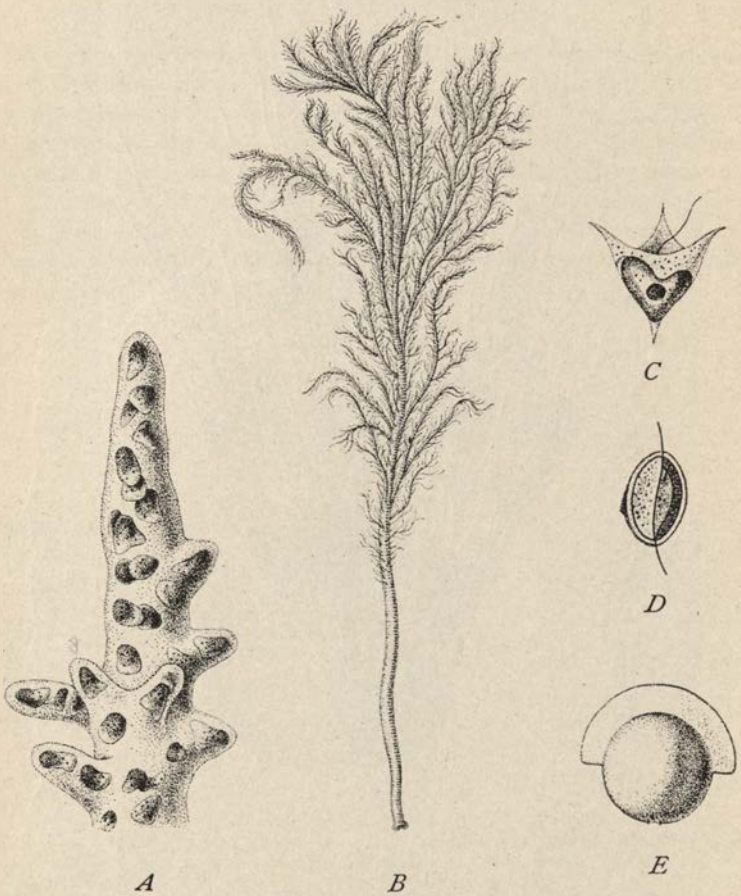

FIG. 127.—*Hydrurus fœtidus*. *B*, character of growth; *A*, end of single branch with monads in the gelatinous matrix; *C*, motile stage of monad; *D*, *E*, side and top view of cyst. (*A*, *C*, *D*, *E*, from Doflein after Klebs; *B*, from Pascher.)

phase. In most such cases reproduction ensues and a Palmella-stage results which is purely facultative in many types, but in some groups, notably in the sub-order Chrysocapsina, the Palmella-stage is dominant, the most extreme case, *Hydrurus*, with its apical growth and method of branching, being highly suggestive of the multicellular algæ (Fig. 127).

In a number of different types the monads lose their chlorophyll and stigmata and, like colorless flagellates, live as saprophytes or by strictly holozoic means. Pascher, Doflein, Franz and others regard such forms as illustrating the probable mode of origin of the Zoömastigoda.

Encystment and reproduction within the cyst is characteristic of the group the cysts being unique amongst the flagellates in having silicious walls. In rare cases, apparently, does the protoplasm of the monad retract from the membrane which then becomes the cyst wall. A more frequent method, according to Scherffel (1911) is the formation of a hollow shell within the ectoplasm of the monad, the shell being entirely closed save for a pore at one pole. The ectoplasm, after variously sculpturing the outer surface of the shell, retires into the pore which is then closed with a silicious stopper (Fig. 5, p. 24). Comparatively few types, however, have been examined and the prevalence of this peculiar method of cyst formation remains to be demonstrated. Within the cyst the monad divides to form two or more spores the germination of which has been observed.

The sub-orders of the group, according to Pascher's classification, are decidedly artificial, being based upon the relative importance of the Palmella-stage in the life history. In the Euchrysomonadina the Palmella-stage is temporary and facultative and the flagella-bearing or motile stage is dominant. In the Chrysocapsina the motile stage is temporary and the Palmella-stage dominant, and in the Rhizochrysidina the flagellum-bearing phase has never been observed, the organisms being placed here rather than with the Sarcodina because of their yellow-brown chromatophores and products of metabolism.

Sub-order I. **Euchrysomonadina.**

Yellow or brown monads with one or two flagella which may be discarded from time to time, pseudopodia taking their place. In some forms the pseudopodia stage represents the fully grown or mature phase of the organism. We recognize five families, three of which—Chromulinidæ, Isochrysidæ, and Ochromonadidæ—are represented mainly by fresh-water forms; but investigation will probably show that there are as many salt-water forms. Two families—Silicoflagellidæ and Coccolithophoridæ—are exclusively marine.

Family 1.—**Chromulinidæ.**—Usually minute forms with a single apical flagellum; solitary for the most part, colony forms rare (one genus, *Chrysosphærella*). They may be either free-swimming or attached, naked or covered by a shell. Pseudopodia formation

characteristic, the flagellum being retained or discarded. Nutrition autotrophic or heterotrophic.

Three subdivisions, here given the value of Tribes, are recognized:

Tribe A. Chrysapsidinæ, Pascher. With undifferentiated chromatophores in the form of a network or in diffuse clumps; stigma present, also contractile vacuole; nutrition holophytic and holozoic; reproduction by longitudinal division; spine-bearing cysts known in one species. One genus only, *Chrysapsis*, Pascher (Fig. 124).

Tribe B. Euchromulininæ, Pascher.—With distinctly differentiated chromatophores and soft naked bodies without definite pellicle; many types with delicate loosely-fitting houses (*Kephyrion*, Pascher), others with thick shells (*Chrysococcus*). Pseudopodia formation is characteristic, the pseudopodia being used for ingestion of solid food bodies and in some cases forming a crown about the base of the flagellum (*Cyrtophora*, etc., Fig. 94, p. 200). In other cases the flagellum is withdrawn and fine branching pseudopodia take its place (*Chrysopyxis*, Stein). Budding frequent.

Tribe C. Mallomonadinæ, Pascher.—This group includes solitary and colonial (*Chrysosphærella*) forms with thick, closely attached membranes with superficial granules (*Microglena*) or silicious plates (*Mallomonas*), the latter bearing, in some cases, long silicious needles (*Mallomonas, Chrysosphærella*). The monads usually have a somewhat complicated vacuole system consisting of an apical, non-contractile vesicle and a few contractile vacuoles scattered here and there about it (Fig. 125). Cysts are known for a number of species but reproduction is very little known.

Family 2. **Isochrysidæ**, Pascher.—Little-known forms; solitary or colonial, with two equal flagella at the apical end of the cell; the individuals secrete a jelly in *Syncrypta volvox* (gelatinous matrix being absent in the colonies of *Synura* and in the band-form colonies of *Chlorodesmus*). Tests with stalks are present in *Stylochrysalis*, and without stalks in *Derepyxis* Stokes, in which the body is attached to the inside of the test by delicate protoplasmic processes.

Family 3. **Ochromonadidæ**, Senn.—Chrysomonads of simple structure with two flagella, one of which, primary, is longer than the other (secondary); they are either solitary or colonial with a more or less plastic body and with a widespread tendency to form delicate loose-fitting tests. The vacuoles are simple and pusules or non-contractile vacuoles are absent.

Sub-family Ochromonadinæ, Pascher.—Here are included the naked, solitary or colonial forms without specialized pellicular structure, membranes or tests. The gelatinous colony forms are the most characteristic especially the relatively large (up to 400 μ) *Uroglena* and *Uroglenopsis* types (Fig. 18, p. 39). These aggregates are so delicate that they go to pieces easily in water mains,

after which the monads disintegrate liberating the contained oil and fat drops which cause offensive odors and tastes (*Uroglenopsis americana*, Calkins). In *Uroglena* the several monads are connected with the interior of the colony by long basal threads. Chromatophores one or two; nutrition holophytic, with tendency in some forms to lose the chromatophores and live as animals.

Sub-family Lepochromonadinæ, Pascher.—These are the house or test-dwelling ochromonads; the tests are extremely delicate with the monads seated at the wider, open ends, or at the base, and with or without a contractile stalk. The tests are frequently complex owing to sculpturing or to superimposed growth rings (*Dinobryon*, *Hyalobryon*, etc., Fig. 126). The genus *Dinobryon* is remarkably rich in forms and is one of the most common genera found in fresh water. Some species are solitary, others colonial with highly divergent types of arboroid colony formation, the bases of the younger tests attached to the inner sides of the older ones. In *Hyalobryon* the tests are attached on the outsides of the older ones (Fig. 19, p. 40).

Family 4. **Coccolithophoridæ**, Lohmann.—This group comprising the smallest forms of marine plankton, is characterized mainly by the peculiar discs of calcium carbonate which make up the shells. The shell pieces are in the form of either solid discoidal plates (discoliths) or of funnel-like structures (tremaliths), the latter perforated by a distinct pore and bearing flattened plates at one or both ends. The separate plates were known long before the organisms which carry them and received the name of "coccoliths." Huxley at one time interpreting them as the skeletal parts of *Bathybius*. The structure of the monads is typical of the Chrysomonadida, with one or two flagella, yellow chromatophores and autotrophic nutrition.

The most complete observations on the Coccolithophoridæ were made by Lohmann, but observations on the mode of reproduction of these forms are lamentably fragmentary and unconvincing. Division through the main axis as in other Chrysomonadida is indicated, and there is some evidence of heteromorphic shells (macrotheca and microtheca, Lohmann). We follow Lohmann in distributing the genera between two sub-families.

Sub-family Syracosphærinæ, with individuals provided with one or two flagella and with shells made up of imperforate calcareous discs.

Sub-family Coccolithophorinæ, with individuals provided with one flagellum and with shells made up of perforated discs which may be simple or provided with hollow tubular processes, pipe-like or trumpet-shape in form.

Family 5. **Silicoflagellidæ**, Borgert.—The genera and species of this Family, found only in the sea, are characterized by the presence

of a simple skeleton of silica in the form of a ring (*Mesocena*, Lemm.) or of a ring with bars. *Distephanus speculum*, Ehr. is associated with Radiolaria as a parasite or a symbiont. It has a single flagellum, yellow chromatophores and a fenestrated silicious skeleton. Little is known about its life history and at one time it was supposed to be a stage in the development of certain forms of Radiolaria (Fig. 128).

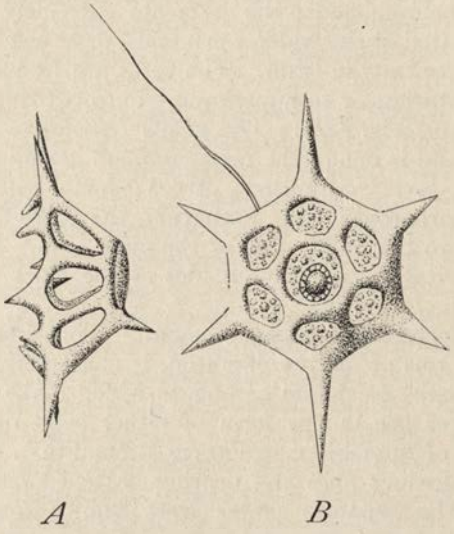

FIG. 128.—*Distephanus speculum*, side (*A*) and top (*B*) views of skeleton. (From Calkins after Borgert.)

SUB-ORDER 2. **Rhizochrysidina**, PASCHER.

A highly artificial group consisting of genera in which no flagella have been described but which move by means of pseudopodia. The temporary nature of the group is indicated by the fact that as soon as a flagellum is observed in any member of the sub-division, that species becomes one of the Euchrysomonadina. It is retained in the classification merely for convenience in holding pseudopodia-bearing forms with yellow chromatophores and no flagella. The genera *Rhizochrysis*, *Chrysidiastrum* and *Chrysostephanosphœra* are naked, while *Stylococcus* and *Lagynion* are test-dwelling with long, thread-like, often single, pseudopodia.

SUB-ORDER 3. **Chrysocapsina**, PASCHER.

While Palmella stages are characteristic of all the Chrysomonadida in this group they become the prevailing stage and flagellated forms are transitory. Here the individuals are enclosed in a gelatinous

matrix in which they reproduce by division forming great colony-like aggregates attached to water plants (Hydruridæ) or smaller planktonic gelatinous masses. A simple contractile vacuole, a single chromatophore, and single or double flagella are typical of the individual monads. The group is rather artificial but very interesting theoretically, with transition forms between motile forms of flagellates and typical plants with apical growth (*Hydrurus*, Fig. 127). Two families are given in Pascher's classification:

Family 1. **Chrysocapsidæ**, Pascher. — Palmella-stages relatively small gelatinous masses of varied form and with no evidence of apical growth. Reproduction by division of monads which become flagellated, leave the gelatinous matrix, swim freely for a time, then lose the flagellum, become quiescent, secrete a gelatinous substance and divide.

Family 2. **Hydruridæ**, Klebs.—Palmella-stage large, up to 30 cm., usually cylindrical in form, and usually branched. The monads are loosely arranged in the gelatinous matrix, a single one forming the apex of each branch. With division of this apical cell one of the products becomes apical while the other contributes to the thickening of the mass. The division products of all cells may at any time develop a single flagellum and swim off in the form of a four-cornered pyramidal flagellate (Fig. 127, *C*) with a single yellow chromatophore and a leucosin mass. Characteristic cysts with silicious walls and stopper are present. One genus and species *Hydrurus fœtidus*, Kirschner (Fig. 127).

Order II. **CRYPTOMONADIDA**, Stein.

The Cryptomonadida, like the Chrysomonadida, are small colored flagellates which rarely measure more than 30 μ (exceptional species up to 80 μ). They differ from Chrysomonadida in having a constant body form with little tendency to form pseudopodia. Many are laterally compressed and show a dorso-ventral differentation due to a median furrow which passes obliquely over the anterior end, resulting in an arched dorsal and a flat ventral side. In some types the furrow leads into a cup- or tube-shape depression which may extend deep within the endoplasm as a cytopharynx.

Flagella are one or two in number and are frequently band-form with a tapering extremity; if two are present one is usually thicker than the other. They are inserted in the furrow or on the ventral wall of the cytopharynx.

The pellicle is simple and delicate in some forms but may be heavy and provided with striations which have been questionably interpreted as myonemes to which contractions of the body are due.

Many forms are colorless but in the group as a whole chromatophores of yellow, brown, blue, blue-green and green color are present.

In some cases these are numerous, small, and discoidal in form; in other cases there are only one or two in the cell in which case they are cup-shape or band-form and laterally placed. The exact nature of the coloring matter is unknown but is generally supposed to be the same as that of the Dinoflagellida. Stigmata in the vicinity of the flagellum-base are common. One or more globular pyrenoids usually enclosed in a shell of amyloid substance, are likewise widely distributed. Trichocyst-like rods in the anterior end, are found in several types, from which on treatment with reagents, gelatinous filaments are thrown out. The nature of these structures and their functions, are entirely unknown. One or two simple contractile vacuoles are present in the majority of forms and are situated in the anterior part of the dorsal side and empty into the cytopharynx.

Longitudinal division occurs usually in the motile stage although it takes place in some cases during resting phases. Palmella-like gelatinous aggregates are characteristic of some forms; such a phase is dominant in one family (Phæocapsidæ) where the aggregates take the form of simple or branched threads. Swarmers having the characteristic cryptomonad structure leave the aggregates for purposes of reproduction. Sexual processes are entirely unknown.

Nutrition may be either autotrophic or heterotrophic; many forms live as saprophytes, many as symbionts (especially the Zoöxanthellæ) and still others are holozoic. Amyloid substances are usually present as inclusions in all types, but starch is confined to the higher types. Fats and oils are occasionally found in some of the colorless forms.

Encysted stages are usually spherical, the cyst walls being made of cellulose. The thickened pellicle of some forms becomes the cyst wall.

The Cryptomonadida are widely distributed in salt water while fresh-water forms are relatively scarce and are never strictly planktonic. Many types thrive in infusions where, as saprophytes, they live upon dissolved proteins of disintegrating plant and animal tissues; some are also holozoic.

Pascher subdivides the Order into two sub-orders which will probably be increased when the life histories of more types of marine forms are better known.

Sub-order 1. **Eucryptomonadina,** Pascher.

Including the motile, flagellum-bearing types which only exceptionally pass into a Palmella-stage.

Family 1. **Cryptomonadidæ,** Stein.—In this group are included the more highly organized forms of the Order. The obliquely truncated anterior end carries the two flagella and in the more differentiated forms there is a distinct cytopharynx. *Chilomonas*

often filled with carbohydrates, and *Cyathomonas* are colorless and live as saprophytes the latter also taking in bacteria, other small protozoa and algæ. Yellow chromatophores are present in *Cryptomonas* and *Chrysidella*, the latter symbiotic with Radiolaria, Foraminifera and other marine animals. *Rhodomonas* has red, *Chromomonas* and *Cyanomonas* blue, *Cryptochrysis* brown or olive green, chromatophores. Reproduction by longitudinal division occurs in the motile stages or during resting phases, the latter sometimes resulting in Palmella-like small aggregates which quickly break up. The life history is practically unknown for any species and the finer cytological structures equally so.

Family 2. **Nephroselmidæ**, Pascher.—Here the characteristic furrow is equatorial thus imparting a bean- or kidney-shape to the cells. The two flagella arising from the furrow are thus apparently lateral, one being directed forward, the other backward.

Sub-order 2. **Phæocapsina**, Pascher.

This group of Cryptomonadida corresponds to the Chrysocapsina group of the Chrysomonadida consisting of forms which are practically in a permanent Palmella-stage, giving rise, however, to flagellated swarmers of the cryptomonad type. The single individuals are sometimes in gelatinous walls from which long thread-like or tubular processes arise (*Nægeliella*), or sometimes in thread-form slightly branched aggregates (*Phæothamnion*). These two types form the basis for the families (1) Phæocapsidæ and (2) Phæothamnidæ.

Order III. **DINOFLAGELLIDA**, Stein.

We follow Doflein in reducing the Dinoflagellida from the value of a sub-class in earlier classifications, to the present position in the Phytomastigoda. The affinities of this well-circumscribed group are apparently more closely with the Cryptomonadida than with any other type of Protozoa. The yellow-brown color of the chromatophores, the peculiar furrows, the nature and positions of the flagella, and the alga-like nature of the series of forms which are permanently without flagella, are characteristics with prototypes or parallels in the orders already considered.

We go farther than Doflein and follow Kofoid and Swezy, not only in placing the Cystoflagellina as a sub-order of the Dinoflagellida, but in removing *Noctiluca* from the cystoflagellina and including it with Gymnodinioidæ. The homologies which Kofoid has recently drawn between the swarm spores of *Noctiluca* and the Dinoflagellida are clearly sustained and there are the same grounds for regarding *Noctiluca* as a Dinoflagellate that there are for considering *Hydrurus* a Chrysomonad because of its temporary flagel-

lated phase, or the Phæocapsidæ as Cryptomonads for similar reasons.

The majority of Dinoflagellida are covered by distinct shells composed of a cellulose-like substance; yet some of them (Gymnodinioidæ) have none; the majority also bear characteristic cross and longitudinal furrows; yet some of them (Adinina) have no trace of furrows. The surface-dwelling forms for the most part have distinct chromatophores; but a number of these, and all of the depth-dwelling types, are devoid of chromatophores. The peculiarities of the group in regard to these structures are so characteristic that if only one is present and the others absent, it is sufficient to classify the organism.

The Dinoflagellida are sometimes associated in classification with the diatoms and desmids (Zygophyta). Many zoölogists still regard them as representing a major group of flagellates (Bütschli for example, divided the Mastigophora into Flagellata, Dinoflagellata, and Cystoflagellata) but with increasing knowledge of life histories and finer structures the prevailing opinion at present is that the Dinoflagellida are more closely related to the Cryptomonadida than to any other group.

While many of the Dinoflagellida are naked, the majority are entirely covered by a definite refractile membrane of cellulose-like substance which is often impregnated with inorganic, frequently calcareous, deposits. In some forms a limited region, the so-called rhombic area (Kofoid), remains unprotected. The test is simple and apparently made up of one piece in *Glenodinium* and *Hemidinium*, or of two valves as in the Adinina and the Dinophysidae, while in the family Peridinidæ they are composed of plates of definite form and arrangement which are frequently areolated and characteristic of the species in each case. These plates are often drawn out into typical horns and processes (*e. g.*, *Acanthodinium* Kof.) while in the Dinophysidæ fin-like ridges and wing-like processes often give rise to fantastic shapes (*Dinophysis*, *Ornithocercus*, etc.).

Furrows on the surface of the cell, foreshadowed in forms like *Nephroselmis* or *Cryptochrysis* of the Cryptomonadida, are highly characteristic of the Dinoflagellates. One of these the girdle, or annulus, is annular, running around the body near the center or excentrically, or following a spiral course. In *Polykrikos* there are several such transverse furrows (Fig. 132). In the corticate forms there is usually a separate girdle plate which follows the contour of the groove while its free edges are often drawn out into ledge-like outgrowths. In some remarkable types the transverse groove is at the apical extremity of the body (*Amphisolenia*, *Dinophysis*, *Amphidinium*, Fig. 70) and a series including these forms with *Prorocentrum* and *Exuviælla* (Fig. 129), suggests the possibility that the Adinina are extreme representatives of a series in which

the groove approaches the anterior end and finally disappears entirely from the cell. In *Prorocentrum* (Fig. 129, *A*) there is an anterior spur-like process about which the transverse flagellum vibrates as though in a groove, while in *Exuviælla* even this spur is absent but the flagellum vibrates in a plane at right angles to the

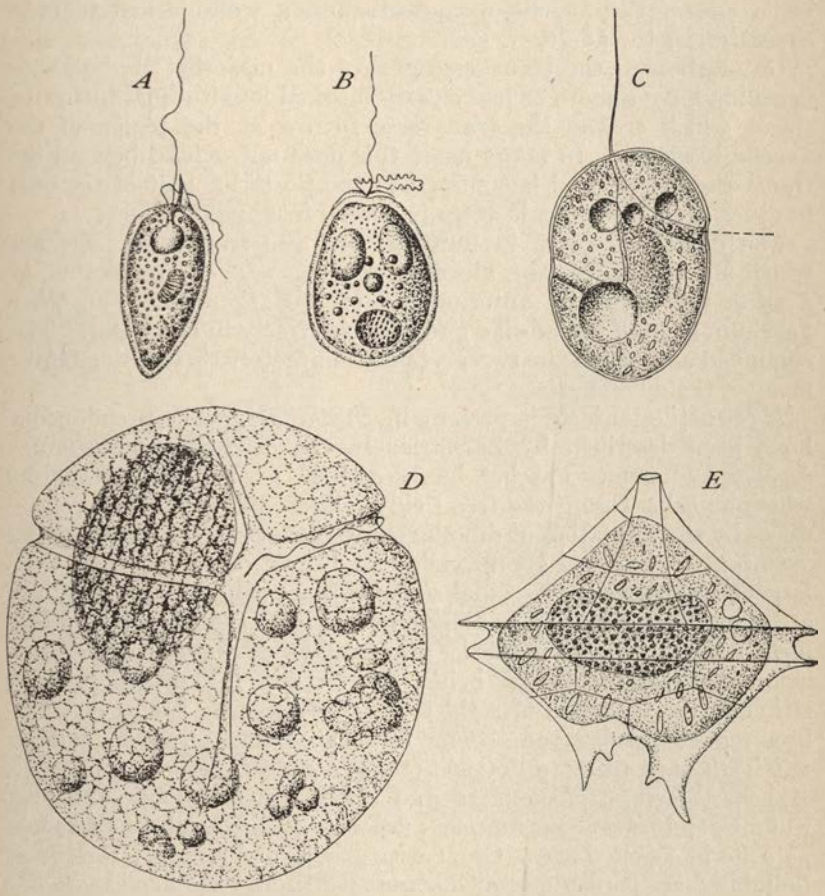

Fig. 129.—Types of Dinoflagellida. *A*, *Prorocentrum micans; B*, *Exuviælla marina; C*, *Gyrodinium ovum; D*, *Gymnodinium sphæricum; E*, *Peridinium divergens.* (*A*, after Bütschli; *B*, to *E* after Calkins.)

long axis of the cell as though in an imaginary furrow (Fig. 129, *B*). It seems highly improbable that such a peculiar mode of flagellar vibration is evidence of a primitive form of Dinoflagellates and more reasonable to regard these types as secondarily simplified organisms derived from the Dinophysidæ, a derivation further evidenced by

the two valves and the median suture, and they are so treated in the following classification.

The marine form *Oxyrrhis marina*, which as Senn was the first to demonstrate, should be placed with the Dinoflagellida, has a rather indefinite groove and two free flagella (Fig. 43, p. 88). The flagellated swarmers of *Noctiluca miliaris* have a similar groove while one of the flagella disappears and a well-marked tentacle characterizes the adult.

In addition to the transverse groove the majority of the Dinoflagellida have a more or less clearly marked longitudinal furrow or sulcus which crosses the transverse furrow at the region of the flagella insertion. In many cases this does not extend beyond the transverse groove and is confined to the posterior half of the cell; in other cases however, it extends nearly from pole to pole.

The flagella are two in number, one the transverse flagellum which may be band-form, vibrates in the groove or, in Adinina, in a circle free from the anterior end of the organism. The other flagellum is more thread-like and vibrates freely in the water. The combined activity of the two gives the characteristic whirling movement of the dinoflagellate type.

A retractile tentacle is present in *Erythropsis* while pseudopodia have been described by Zacharias in the case of *Gymnodinium zachariasi*, Lemm. Pascher has described an amœboid organism which he calls *Dinamœba* (see Leidy) which, like *Noctiluca*, reproduces by the formation of dinoflagellate-like swarmers (dinospores).

Chromatophores of green, yellow or brown color and from two, large, to many small, discoidal structures are generally present, but are absent here and there in all groups and in all species of the bathymetrical group. Nutrition is autotrophic or heterotrophic, the chlorophyll-bearing forms being autotrophic for the most part although the ingestion of solid food substances by such types has been repeatedly observed. Some forms are parasitic, *Gymnodinium pulvisculus* according to Pouchet (1886) is an ectoparasite on *Salpa*, *Appendicularia*, *Siphonophora* and other marine pelagic animals, while *Gymnodinium parasiticum*, according to Dogiel, is an endoparasite in copepod eggs. One entire group furthermore—the Blastodinidæ—are parasitic (see Chatton, 1920).

Stigmata, also, while not universal, are widely distributed and in some cases are accompanied by a crystalline amyloid structure which appears to function as a lens (Fauré-Fremiet; Schütt).

Vacuoles of a somewhat different type from the usual contractile vacuoles are always present. They are filled with a clear fluid and appear to have definite walls, opening to the outside in the region of the flagella fissure. Two types of these vacuoles are usually present, one, called the collecting pusule by Schütt may be surrounded by a ring of small casual vacuoles (Kofoid) and resemble

the more common forms of contractile vacuole; the other, called the sac pusule, is very large and tends to assume the general shape of the cell, opening by a fine canal to the outside. Their functions are problematical but they are generally regarded as serving an hydrostatic function.

In one group of forms, the Phytodinidæ, the characteristic structural features, furrows, flagella, etc., are absent, the organisms, like unicellular plants, either floating, lying freely on the bottom or attached to it. The nucleus and chromatophores, however, are of

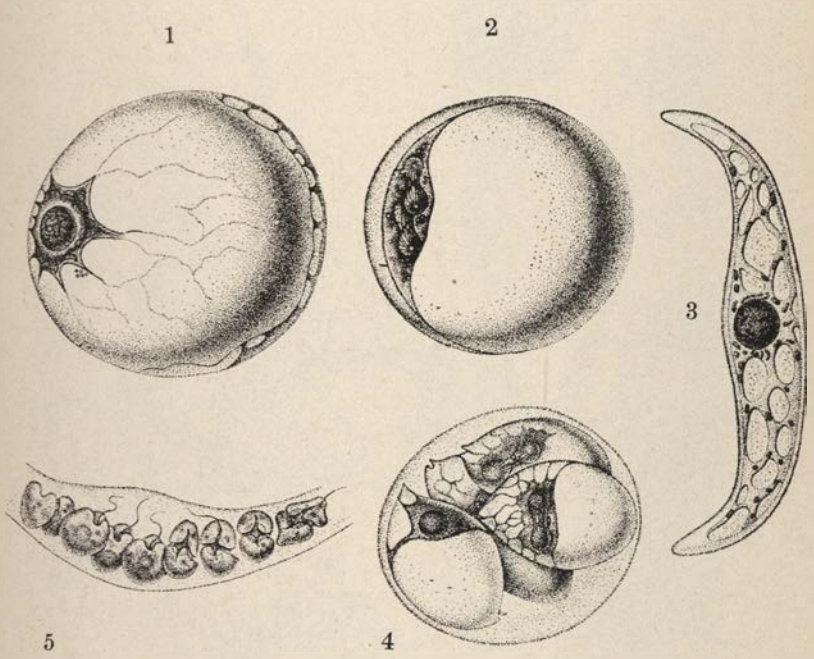

FIG. 130.—*Gymnodinium lunula* (Schütt). Nuclear division in large pelagic cysts (1 and 2); 3, so-called "horned" cyst; 4, four products of division of 2; 5, eight *Gymnodinium*-like products of horned cyst. (From Doflein after Dogiel.)

the dinoflagellate type and Klebs, and later students of the group, have no hesitation in placing them with the Dinoflagellida. This is supported by numerous transitional forms from the typical members of the order; some of these are similarly quiescent, devoid of flagella, but possess the characteristic furrows; others possess flagella and furrows for a period in the life history but lose them and become quiescent and plant-like. Some types of the latter secrete gelatinous coatings (*Glœodinium*), others form peculiar reproductive cysts "horned cysts" (Fig. 130). These aberrant types of Dinoflagellida illustrate the same phenomenon of motile and quiescent

stages characteristic of the Chrysomonadida and the Cryptomonadida.

Reproduction by division occurs either in the motile or resting stages in different cases. Freely moving Gymnodinidæ divide in

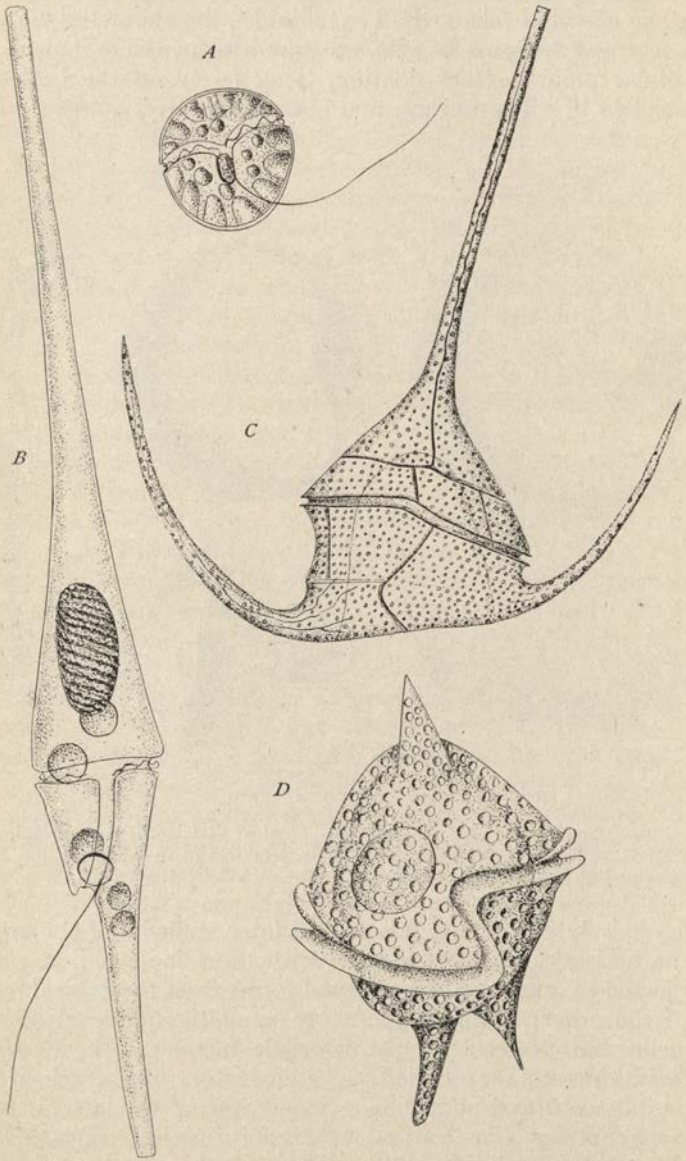

FIG. 131.—Types of Dinoflagellida. *A*, *Glenodinium cinctum*; *B*, *Ceratium fusus*; *C*, *Ceratium tripos*; *D*, *Gyrodinium* sp.

the long axis much like any other flagellate; shelled forms, like *Ceratium*, have a more involved division process by reason of the hard covering of plates. Here the division plane passes through the sutures between certain definite plates (Fig. 131). The daughter cells in some cases remain attached to form peculiar and characteristic catenoid colonies and the peculiar form *Polykrikos* is regarded as such a colony which has become permanent (Fig. 132). Division

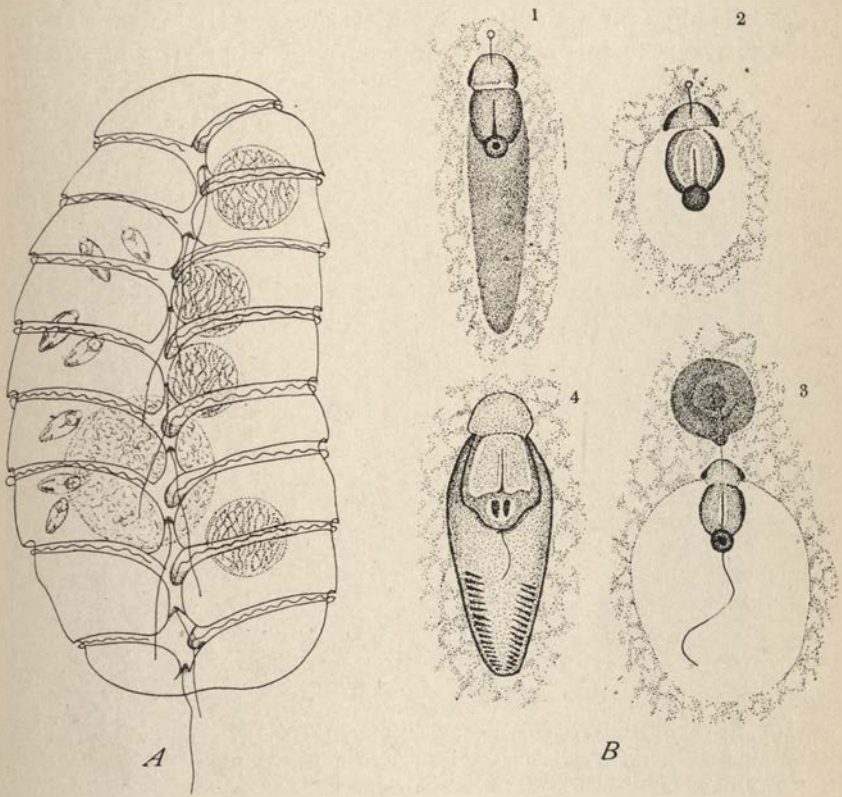

FIG. 132.—*Polykrikos schwartzi* and development of its nematocysts. (After Chatton.)

in resting phases differs according to the species and varies in permanent and temporary resting conditions; sometimes it occurs within the parent shell, in other cases the outer shell is discarded before division.

Multiple division is widespread especially in quiescent reproductive forms protected by gelatinous membranes or cysts; the latter are sometimes drawn out into peculiar crescentic capsules within which the individual divides to form several products. *Gymnodi-*

nium lunula (Fig. 130) according to Schütt is found in the plankton as a large cyst-like organism, not unlike *Noctiluca*, containing a single nucleus. This divides four times and 16 daughter cells are formed each of which develops into a "horned cyst" and gives rise to 8 small Gymnodinium-like products (Fig. 130, 1, 2, 3, 4, 5). Schütt's inference that these ultimate products are gametes which conjugate and give rise to the spherical cysts, has not been confirmed. Conjugation stages in *Ceratium hirundinella* have been described by G. Entz (1910), and by Zederbaur (1904), which recall the sexual processes of the desmids, the zygote according to their

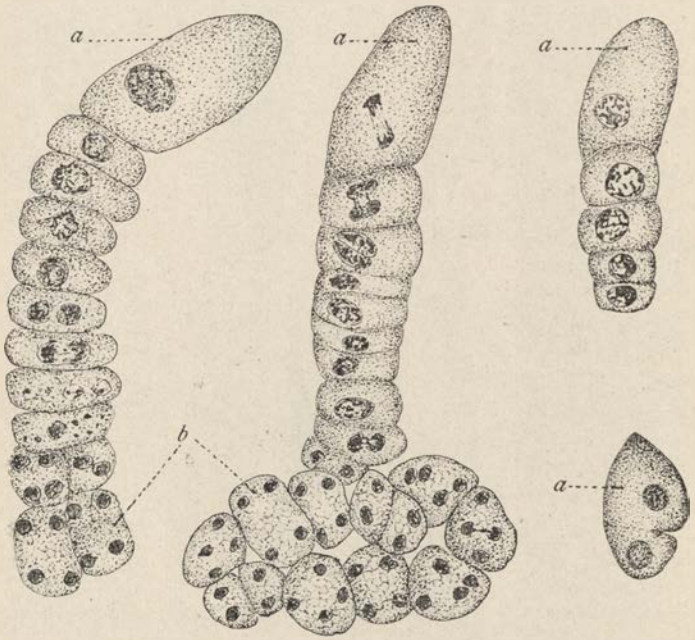

FIG. 133.—*Haplozoön clymenellæ*, regarded by Chatton as a parasitic dinoflagellate; (*a*) primary attaching individual which gives rise to a chain of cells terminating in cells with four nuclei (*b*). (After Calkins.)

interpretation giving rise to the characteristic "four-horned cyst." The great variability, and the frequency of pathological conditions in *Ceratium* make this so-called conjugation process questionable.

The Blastodinidæ are all parasitic and have lost most of the characteristic dinoflagellate structures, the affinities being indicated, as in *Noctiluca*, by the structure of the spores (dinospores). Some are external parasites on appendages and gill filaments of pelagic animals; on animals, on diatoms (*Paulsenella*), or on eggs of copepods, etc. Others are internal parasites of digestive tract or cœlom (*Syndinium*). Some types (*Apodinium*, *Parapodinium*,

etc.) have attaching trunks or peduncles recalling the epimerites of gregarines; others are sessile, while still others are unattached cœlozoic parasites of body cavity or gut. A curious Cestode-like form, found in the intestines of maldanid annelids, was discovered by Dogiel (1907) and described as a Mesozoön under the name of *Haplozoön* (Fig. 133). Chatton (1920), however, has shown that its affinities are with the Blastodinidæ and particularly with Blastodinium.

The Dinoflagellida are distributed amongst three sub-orders—Diniferina, Adinina and Cystoflagellina.

Sub-order 1. **Diniferina**, Bergh.

The majority of forms with characteristic dinoflagellate structures are placed here. One or more transverse furrows bearing flagella are typically present, and the longitudinal flagellum is usually directed backward. Three Tribes are recognized: (1) Gymnodinioidæ; (2) Perididinioidæ, and (3) Amphilothioidæ.

Tribe A. Gymnodinioidæ, Bergh, Em. Poche.—As the name indicates these interesting forms are either naked or provided with delicate cellulose coverings which are not laid down in plate form. In respect to furrows, flagella, and general structure they conform, even when naked, to other members of the sub-order. They are usually somewhat dorso-ventrally flattened and provided with many small discoidal green or yellow chromatophores although some species are colorless. Stigmata are occasionally present, located in or near the longitudinal furrow. Nutrition is either autotrophic or heterotrophic (saprozoic, holozoic, or parasitic) and reproduction usually takes place during encystment, rarely in the motile stages.

In *Hemidinium*, Stein, the cross furrow does not run entirely around the cell, but, with the flagellum, stops in the center of the dorsal surface. *Gymnodinium* is frequently colorless, taking in solid food and, in some species (*Gymnodinium zachariasi*, Lemmermann), forming branched pseudopodia. Other species (*Gymnodinium parasiticum*, *Gymnodinium roseum*) are parasitic on the eggs of copepods. Other genera are peculiar in having only a short free-swimming period after which they pass into an alga-like resting phase. Such forms as *Cystodinium* and *Gymnodinium lunula* are strikingly suggestive of the Phytodinidæ in having motile stages lasting from only a few minutes to an hour or so, while in *Hypnodinium* no motile stage whatsoever is known. It is in the life history of these types that the so-called "horned cysts" are found (see p. 271). The genus *Glenodinium* differs from most of the Gymnodinidæ in having a definite but delicate and non-articulated shell (Fig. 131, *A*).

Oxyrrhis, Pouchetia, Erythropsis, Polykrikos, etc., are colorless types with various structural peculiarities which distinguish them from other dinoflagellates. *Oxyrrhis marina* has a rudimentary furrow and no encircling flagellum; *Erythropsis* has a definite tentacle capable of extension and retraction, while it, together with *Pouchetia,* has a huge stigma provided with a distinct lens; *Polykrikos* has several transverse furrows with flagella and one (*P. auricularia,* Bergh) or several (*P. schwartzi,* Bütschli, *P. kofoidi,* Chatton) axial flagella. This genus also is remarkable in having both trichocysts and nematocysts the latter especially in *P. schwartzi,* have been studied in detail by Chatton (Fig. 132).

Tribe B. Peridinioidæ, Bütschli, Bergh.—*Family* 1. *Peridinidæ* are found both in fresh and in salt water, many of them being deep-sea forms. Green-yellow or brown discoidal chromatophores are usually present in large numbers but many deep-sea species are entirely colorless. The main characteristic of the family is the nature of the shell which is always made up of distinct plates the number and arrangement of these plates affording a basis for generic and specific differences. The transverse furrow is covered by a single annular girdle plate or cingulum which serves as a dividing line between an anterior and a posterior part of the organism. The former portion, called the epitheca, consists of apicals and dorsal intercalaries and the latter, called the hypotheca, consists of precingulars, postcingulars and antapicals while the region of the flagella origin is covered by one or more delicate and thin plates forming a region which Stein called the "oral fissure" (Mundspalte) and which Kofoid calls the "ventral area."

Certain plates of both parts of the shell may be developed into characteristic longer or shorter processes or horns (Fig. 131) those from the hypotheca being usually solid while the single horn from the epitheca is hollow and the distal end is open forming the apical pore (Fig. 131, *B, C*). The antapical horns are often provided with ribs, fins, or wings which are frequently developed asymmetrically. The living organisms appear to have alternate periods of active movement and of rest and according to Kofoid, when the flagella are at rest the specific gravity of the shell and body causes the organism to sink from at or near the surface to greater depths. By reason of the asymmetry due to the unequal development of horns, fins and wings, the cell turns in its descent in such a manner that the resistance to the water is increased by exposure of the broader surfaces and sinking is correspondingly retarded. Such excrescences on the shell are thus interpreted as organs of flotation and asymmetry as an adaptation whereby forward movement is not impeded while too rapid sinking is.

In many species, *e. g., Peridinium divergens,* the plates are separated by rather wide bands of intercalary striæ which, as Bütschli

interpreted them, are zones of growth occurring after the plates are formed. Schütt on the other hand regards them as special ribs or thickenings for the purpose of strengthening the shell between the plates. Kofoid dissents from these views and suggests that the intercalary striæ are modified pores or canals lying in the wall and may be adaptations for further communication between the protoplasmic body and the environment. Many of the Peridinidæ have shells which are more or less uniformly perforated by fine canals and, although the inner openings of these pores have not been definitely made out, it is probable that they serve the same function.

The region about the flagellum pore is covered by plates of more delicate type than those of the rest of the shell and the plates are more difficult to make out but there appear to be four such plates in the species of Peridinium, one of which, the rhomboidal plate, may be larger than the others, and this plate, in the genus *Ceratium* becomes the main protection of the oral region.

The flagellar pore is an opening through the thecal wall sometimes with thickened walls which form a tube running a short distance into the inner protoplasm. In many cases both flagella emerge from one such pore but in a large number of forms there are two flagellar pores, one for each flagellum (Fig. 132). Schütt regards the single pore as more primitive.

Family 2. **Dinophysidæ,** Stein, Bergh.—Plates, characteristic of the Peridinidæ are here absent. The shell also, is built on quite a different plan of structure, agreeing with that of the Adinina in consisting of two lateral valves united in a sagittal suture with interlocking teeth, and passing vertically through the longitudinal groove or sulcus. The transverse groove-annulus is high up on the body and is often provided with wide lamellæ. The epitheca and hypotheca, therefore, are widely different in size. In *Amphisolenia* the former becomes almost rudimentary and suggests a transitional form to the Adinina. Asymmetry is characteristic and frequently leads to fantastic form relations. Phæosomes or peculiar brownish masses, are regularly present and brown or yellow chromatophores are often present although many representatives of the family have none at all. They are confined mainly to the warmer seas.

Family 3. **Phytodinidæ,** Klebs.—This family, created by Klebs as a subdivision of the Diniferida, includes forms which more nearly resemble plant cells than Protozoa. With the exception of the yellow-brown chromatophores, radial protoplasmic structure and cellulose walls, there are few Dinoflagellate characteristics. They lack both longitudinal and transverse grooves and have no flagella. Reproduction occurs by simple division, the daughter cells either separate by rupture of the cellulose wall (Phytodinium) or form *Glœocystis*-like colonies. In the latter case thick gelatinous mem-

branes are formed consisting of alternate cellulose walls and gelatinous substance (*Glœodinium*), while *Stylodinium* possesses a gelatinous stalk. Swarmer stages are entirely unknown.

Sub-order 2. **Adinina,** Bergh.

These very interesting forms possess two flagella but no furrows. The body is enclosed in a bivalved shell composed of a substance similar to cellulose. The right and left valves come together in a median sagittal suture as in the Dinophysidæ but interlocking teeth are absent. An aperture between the valves at the anterior end serves as an outlet for the flagella, one of which extends freely in the water while the second, corresponding to the annular flagellum of other Dinoflagellida, after emerging from the shell, bends sharply and vibrates in a circle around the base of the first flagellum (Fig. 129, *B*). This condition is foreshadowed in the genus *Amphidinium* where the epitheca is a mere knob (Fig. 70, p. 136). In *Prorocentrum* the anterior spine-like horn may represent the reminiscence of such a modified epitheca (Fig. 129, *A*). In *Exuviælla* even this remnant is absent.

The yellow chromatophores are band-form and usually two in number enclosing pyrenoids. Division is said to take place in the sagittal plane, each daughter cell retaining one of the parent valves and regenerating a second. The typical genera are *Prorocentrum*, *Haplodinium*, and *Exuviælla*.

Sub-order 3. **Cystoflagellina,** Haeckel.

The systematic position of the two genera *Leptodiscus* and *Craspedotella* which are usually included in the Cystoflagellida, is not yet clearly established. *Noctiluca*, formerly the type genus of the group, is now definitely recognized as a dinoflagellate. Stein, on the basis of structure of the swarm spores, early recognized the possible relationship but Bütschli, followed by the majority of later writers, regarded it as sufficiently distinct to justify its inclusion in an independent group. Kofoid (1920) has given further evidence of its Dinoflagellate affinities.

Noctiluca is spherical, gelatinous in consistency, and from 1 to $1\frac{1}{2}$ mm. in diameter. The protoplasm is massed at one pole with strands passing to the periphery in all directions giving a plant-like or parenchymatous appearance to the cell. Shell and furrow characteristic of the Dinoflagellates are entirely absent. A peculiar, external, "peristomial" apparatus lies above the denser protoplasmic mass. This consists of a collar-like protuberance from the floor of which a minute flagellum, a cross-striped powerful tentacle, and a curious roughened structure called the "tooth" arise.

A large, single nucleus more like that of Dinoflagellates than other protozoan nuclei, undergoes a characteristic process of mitosis during division (Fig. 52, p. 101).

Order IV. **PHYTOMONADIDA**, Blochmann.

The green flagellates included in this order are the most plant-like of all Protozoa. Their closest affinities are probably with the Chlorophyceæ through the algæ. The chromatophores are colored with grass-green chlorophyll while hematochrome (karotin) in some cases masks the green. Nutrition therefore, is typically holophytic except in the Polytomidæ where chromatophores are frequently absent and nutrition is saprozoic. As a result of their method of nutrition assimilation products are almost invariably starch. The chromatophores are usually single, large, cup-shape, and fill the greater part of the cell, while distinct pyrenoids are usually present.

The individual monads are small, spheroidal, or spindle-shape organisms with membranes of cellulose or pektine and are never metabolic. Flagella vary in number from 1 to 2 (rarely 4 to 8) and protrude from the anterior end through a definite pore in the membrane. The vacuole system is simple, usually consisting of a number of contractile vacuoles grouped at the anterior end. The nucleus is distinct, usually near the center of the cell and divides in such a plane that longitudinal division of the cell is assured. This occurs within the cellulose membrane which is left as an empty shell when the daughter cells emerge. Repeated divisions frequently occur in which case the successive division planes are at right angles to one another. Palmella-stages, either intermediate or terminal in the life history, are frequent while colony formation is highly characteristic, particularly in the Volvocidæ. Encystment stages in which the monads retract from the cellulose membranes are widespread.

Sexual processes are highly characteristic and involve more or less specialized sexually differentiated individuals or colonies with isogamous or anisogamous gametes (see p. 503).

Family 1. **Polyblepharidæ**, Dangeard.—These forms differ from the majority of other Phytomonadida in the absence of cellulose membranes and in the tendency to metaboly. They represent connecting forms with other colored flagellates and are more generalized than other members of the group. This is shown by the absence of sexual processes and by the larger number of flagella possessed by individuals. Typical genus *Pyramimonas*.

Family 2. **Phacotidæ**, Poche.—Phytomonads with a bivalve shell, or at least a membrane which splits easily to form two lens-like halves as in *Phacotus lenticularis*. Two flagella pass through a canal to the outside. Chromatophores and stigmata are character-

istic. Reproduction occurs by division within the shell the valves being pushed apart by swelling of the gelatinous contents when the daughter cells are mature. Fertilization by anisogamy.

Family 3. **Polytomidæ**, Poche.—These are colorless saprozoic forms ellipsoidal in shape (*Polytoma*) or with a truncated anterior

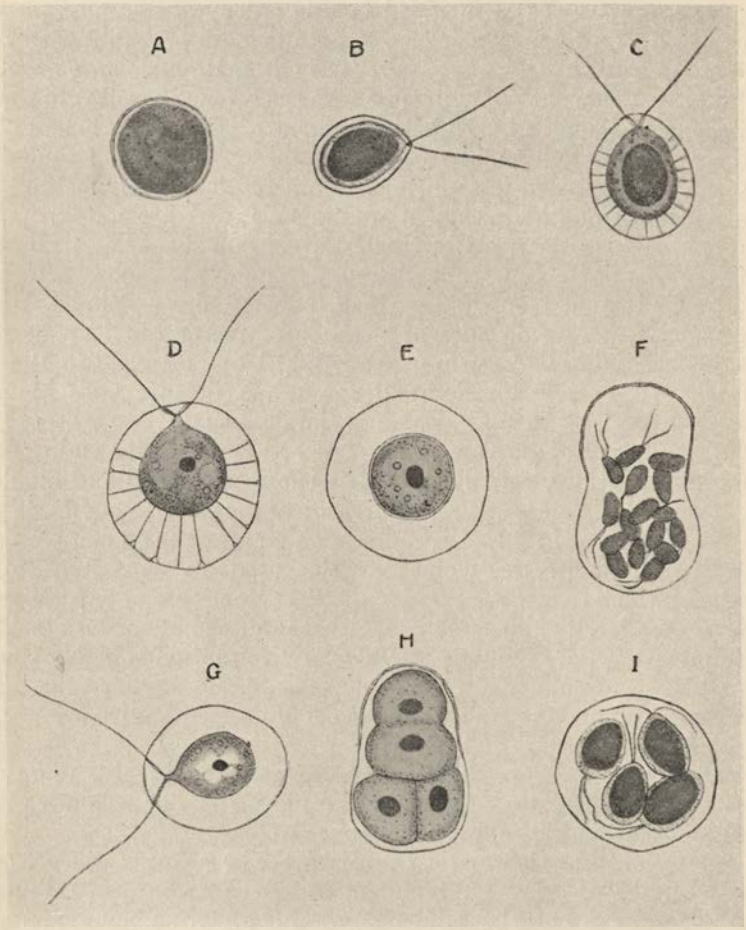

FIG. 134.—*Sphærella lacustris*. (After Hazen.)

end recalling the Cryptomonadida (*Parapolytoma*). Starch is formed and collected at the posterior end of *Polytoma* but is absent in *Parapolytoma*. Reproduction by division into two or four daughter cells, the second division in the latter case being transverse. Blepharoplasts and flagella are lost and reformed at each division (Jameson).

Family 4. **Chlamydomonadidæ**, Bütschli.—These are small, solitary flagellates, with from 2 to 4 flagella, 1 green chromatophore, red stigma and with 1 to several pyrenoids or with none at all. Reproduction occurs by longitudinal or transverse division in either resting or motile phases (Fig. 134). If in the resting stage large gelatinous aggregates (Palmella-phase) result. Such aggregates of *Chlamydomonas nivalis* (*Hæmatococcus nivalis*) under con-

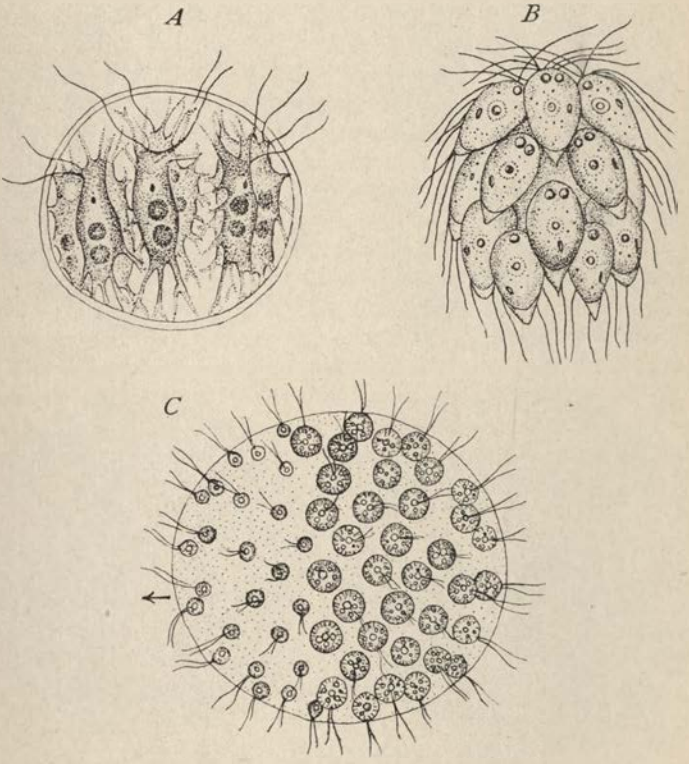

Fig. 135.—Types of colonial flagellates. *A*, *Stephanosphæra pluvialis*, after Kühn; *B*, *Spondylomorum quarternarium* from Doflein after Stein; *C*, *Pleodorina californica* from Doflein after Chatton.

ditions of lack of nitrogen and phosphorus in the environment give rise to the phenomenon of "red snow" or "blood snow" in northern or alpine regions. Fertilization occurs by the permanent union of iso- or anisogametes.

Family 5. **Volvocidæ**, Ehrenberg.—The organisms included in this family represent the highest type of development of the plant-flagellates. They are colonial and the constituent cells are frequently differentiated for different functions. Individually the

single cells of the colonies are built on the same plan as the Chlamydomonadidæ with 2 (rarely 4) equal flagella, non-metabolic body a simple contractile vacuole, chromatophores, stigmata and cellulose walls.

Asexual reproduction occurs in the individual cells within the cellulose membranes and results, usually, in the formation of a new

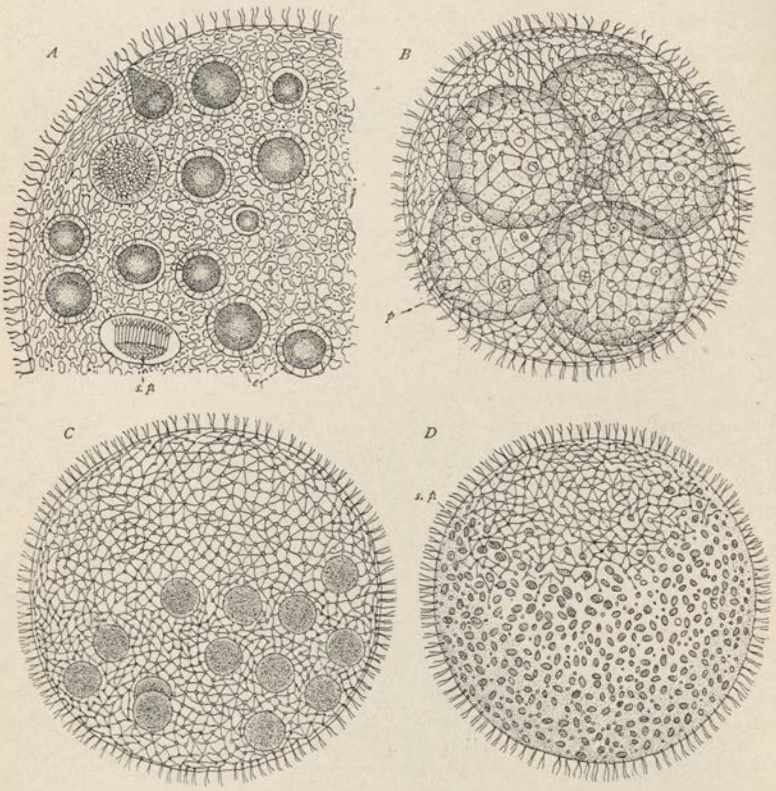

FIG. 136.—*Volvox globator* (*A*) and *V. aureus* (*B*, *C*, *D*). *A*, Sexually mature colony with eggs (*e*, macrogametes), and spermatozoids (s.p.); *B*, asexual colony with young agamous daughter colonies; *C*, female colony with macrogametes; *D*, male colony, with many bundles of spermatozoids (microgametes). (From Oltmanns.)

colony from each cell. In some cases all individuals of the colony thus reproduce the whole (*Gonium*, *Spondylomorum*, etc.) but in others the individual cells of the colony are differentiated into "somatic," and "generative" cells, the latter alone reproducing (*Pleodorina*, *Volvox*, etc.).

Starting with the genus *Spondylomorum* of the preceding family, the different colonial aggregates form a series of increasing com-

plexity culminating in the genus *Volvox*. *Spondylomorum* is a loose aggregate of 16 cells without gelatinous matrix. These have 4 flagella, chromatophores, vacuole, stigma, etc., and are arranged in four alternating rows with the sharp posterior ends pointing in the same direction (Fig. 135, *B*). Reproduction is simultaneous in all 16 cells and 16 daughter colonies result. Sexual processes are unknown.

The genus *Gonium* is more specialized. The 16 cells of *G. pectorale* and the 4 cells of *G. sociale* are embedded in a gelatinous matrix in such a manner as to form a flattened plate of cells with the flagellated ends all turned in the same direction (Fig. 3, p. 21). Here also each of the constituent cells forms an entire colony upon reproduction. Fertilization by permanent union of isogametes is characteristic; an encysted red-colored zygote is formed which germinates after a period of quiescence. The 8-celled globular colonies of *Stephanosphæra* have a similar life history (Fig. 135, *A*).

Pandorina, *Eudorina* and *Platydorina* are colonies of 16 or 32 cells, the first 2 globular, the last a flattened plate with characteristic form (Fig. 3, p. 21). In *Pandorina*, the 16, or rarely 32, cells are pressed together in the center of the colony; in *Eudorina* they are distributed equally throughout the gelatinous matrix. Anisogamy appears to be facultative in *Pandorina* but obligatory in *Eudorina* where the colonies are sexually differentiated some (male) giving rise only to microgametes in bundles of 64, others (female) forming only macrogametes. Here also the encysted zygotes are colored red.

Pleodorina and *Volvox* finally are colonies with permanently differentiated cells (Fig. 135, *C* and Fig. 136). The validity of the genus *Pleodorina* is questioned, Fritsch and Takeda (1916) for example, regarding it as a variety of *Eudorina* which shows all transition stages from a typical *Eudorina* to the conditions described in the original *Pleodorina illinoisensis*, Kofoid (Crow, 1918).

Order V. **EUGLENIDA**, Stein.

The Euglenida comprising the fifth order of Phytomastigoda include some of the largest and most common types of flagellates. They are usually spindle form with a conspicuous periplast which may be variously sculptured or striated, while keel-like processes are occasionally present (*Petalomonas*, *Tropidoscyphus*). A gelatinous mantle in some cases is secreted (*Colacium*) and shells or tests in others; these are soft and gelatinous in *Ascoglena*, rigid and frequently ornamented with papillæ, striations and spines in *Trachelomonas*. Metaboly is characteristic although the phenomenon is unknown for some genera and varies greatly in different species. Spiral markings of the periplast are frequent and torsion

of the cell is highly characteristic of several species (*Euglena tripteris, Euglena oxyuris, Euglena spiroides, Phacus longicauda,* etc.).

The majority of Euglenida possess a definite cytostome from the base of which a flagellum emerges (*Urceolus, Scytomonas,* etc.). In *Entosiphon* a protrusible tube runs deep into the endoplasm. Defecation areas are also present in *Euglenopsis* and *Anisonema.*

Flagella of the Euglenida afford the best material for the study of this type of motile organ. They are frequently large and show the axial fibril and the elastic sheath with great clearness. The majority of forms have only one but two are not uncommon. If two are present they may be of equal length and directed forward (*Eutreptia*) or one may be accessory and very short (*Distigma, Sphenomonas*) or drawn out into a long trailing flagellum (*Notosolenus, Anisonema, Tropidoscyphus, Heteronema, Entosiphon,* etc.).

A complex vacuole system is characteristic. This consists of a reservoir with canal opening into the cytostome and one, two or more contractile vacuoles surrounding the reservoir and emptying into it.

Chromatophores are present in Euglenidæ but absent in the other two families. They are usually discoid, cup-shape, band-form or stellate in shape and may or may not contain pyrenoids. They normally form green chlorophyll in the light, or the green color may be masked by hematochrome (karotin).

Reserve products of nutrition in the form of paramylum and oil are also characteristic. The former apparently increase and decrease in size according to the activity and environment of the cell; these are quite diverse in form but the same type is apparently invariable in the same species, hence they form a suitable means of diagnosis for different species. Stigmata likewise, are common in the Euglenida although they may also be present in colorless Euglenids (*e. g., Astasia ocellata,* Khawkine, *Euglena quartana,* Moroff).

Of the three families Euglenidæ, Astasiidæ, and Heteronemidæ, the first is more commonly represented by fresh-water species. They occur in all waters, Astasiidæ and Heteronemidæ more in infusions and in stagnant pools. Very little reason is to be found for distinguishing a separate family Peranemidæ from the Astasiidæ while colorless forms with two flagella are included in the present family Heteronemidæ.

Family 1. **Euglenidæ**, Stein.—Medium-sized organisms of spindle shape or flattened form and with widely distributed tendency to metaboly. The majority have spiral striations or twisted body. Flagella usually 1 in number, (2 in *Eutreptia*), emerge from the cytopharynx. Chromatophores generally present but may be absent in questionable forms (*Euglena quartana*); similarly with stigmata, while paramylum and oils are usually present. In one genus (*Ascoglena*) the cell occupies an attached gelatinous cup. The

chlorophyll, is green or red (hematochrome) in color. Reproduction by longitudinal division during free-swimming or quiescent periods. Fresh and salt water, more commonly the former.

Family 2. **Astasiidæ**, Bütschli.—Here we include all colorless forms of Euglenida having only one flagellum. The body is usually ellipsoidal, or swollen at the posterior end and metaboly in characteristic but not invariable. The periplast is usually conspicuous and may be ornamented by one or more ridges (carinæ) as in *Petalomonas*. A cytopharynx system is similar to that in Euglenidæ and a stigma is present in at least one exceptional species (*Astasia ocellata*). Movement by creeping or free-swimming; when creeping the flagellum is usually extended straight ahead, the tip, or free end of the axial filament alone in vibration. Reproduction as a rule is by longitudinal division while free-swimming.

Family 3. **Heteronemidæ.**—Colorless forms of Euglenida with 2 flagella, 1 usually directed posteriorly. The vacuole system is complex, stigmata are absent but paramylum is usually present. Body ellipsoidal sometimes metabolic but more often rigid with thick periplast which is often ornamented with longitudinal or spiral ridges. Movement creeping and free-swimming.

Order VI. CHLOROMONADIDA, Klebs.

A very artificial group of comparatively rare forms with obscure affinities. The vacuole system is similar to that of the Euglenida but with starch, grass-green chromatophores of discoidal form, in chlorophyll-bearing types. The cortical protoplasm easily becomes vacuolated, and pseudopodia are present in one genus (*Thaumatomastix*). Trichocyst-like rods are present in *Gonyostomum*. Little is known about their life histories.

Class II. ZOÖMASTIGODA, Doflein.

The animal flagellates have no chromatophores, no chlorophyll and no paramylum granules. The vacuole is a simple vesicle, and cortical differentiations are less extensive and of a different type from those of the Phytomastigoda. On the whole the group includes small forms of relatively less complexity than those of the preceding class and other forms of remarkably great complexity. In one respect particularly, the group shows more complicated differentiations than do the plant flagellates. This has to do with the kinetic and locomotor apparatus, where specializations are complex, especially in the parasitic types. In other respects the animal flagellates parallel the types already outlined, especially as regards form of the body, number and specialization of flagella, and life history. Colony forms are common, particularly the branching

arboroid types; encystment is practically universal; reproduction is by longitudinal division while free-swimming or encysted, or by multiple division whereby a number of individuals are formed from a single cell (somatella) within the membrane of the original cell replacing a cyst wall. Nutrition is holozoic, saprozoic or osmotic (parasitic) and with the parasitic forms, the effects on their hosts vary from none at all to high mortality.

Fertilization processes are practically unknown and the few observations extant have never been confirmed.

Classification of the more primitive forms is still in a tentative phase. Affinities are obscure in most groups and although some appear to be natural others include heterogeneous forms which will be sorted out as observations on life histories accumulate.

Thus the general group of Protomonadina of Blochmann, Doflein, and others, may include representatives of closely related forms which in time will be recognized as sufficiently characteristic to merit independent places in classification. This appears to be the case with the Trypanosomatidæ where the life histories indicate a close relationship of the genera *Herpetomonas, Leptomonas, Leishmania, Crithidia* and *Trypanosoma;* or the group of collar-bearing flagellates included in the earlier families Choanoflagellidæ and Phalansteriidæ. These we raise to the value of sub-orders and give the same value to the pseudopodia-bearing forms with permanent flagella (Pantastomina), otherwise the classification adopted here agrees closely with that used by Doflein, recognizing his Cystoflagellata, however, as Dinoflagellates, and we group the animal flagellates into four orders: Pantastomatida, Protomonadida, Polymastigida and Hypermastigida.

Order I. **PANTASTOMATIDA** (Pantastomina of Minchin).

The ability to protrude pseudopodia is widely spread amongst flagellates but this affords little justification for including such forms with the Sarcodina. The formation of lobose pseudopodia is an expression of the physical consistency of the cell protoplasm and may occur in cells throughout the animal kingdom. The possession of a flagellum throughout life is a distinct characteristic and while such forms may be considered, with *Nägleria*, as transitional forms to the Sarcodina they are nevertheless to be classed as flagellates while *Nägleria* with equal reason is to be classed with other Amœbida in which the temporary flagella are regarded as reminiscent structures. One argument, however, which may be brought against this procedure is the life history of *Mastigella vitrea*, which, as worked out by Goldschmidt (1905) includes chromidia formation and fertilization phenomena which are undeniably similar to those occurring in rhizopods.

The pseudopodia may be axopodia or lobopodia, the axial filaments and radiating pseudopodia of the former resembling the motile organs of the Heliozoa. The lobopodia may be finger-form (*Mastigamœba, Mastigella*) or ray-like (*Actinomonas, Pteridomonas*, etc.), and are frequently limited to either anterior or posterior end.

Flagella vary in number from one to many and dimorphism occurs in some forms (*Pteridomonas, Cercobodo, Bodopsis*). Vacuoles are invariably simple and either fixed or migratory as in *Amœba*.

Nutrition is holozoic, or occasionally saprozoic; solid particles are ingested at any part of the body in some forms; in *Dimorpha* the algal cells serving as food are killed by the pseudopodia and seized by short pseudopodia from the body.

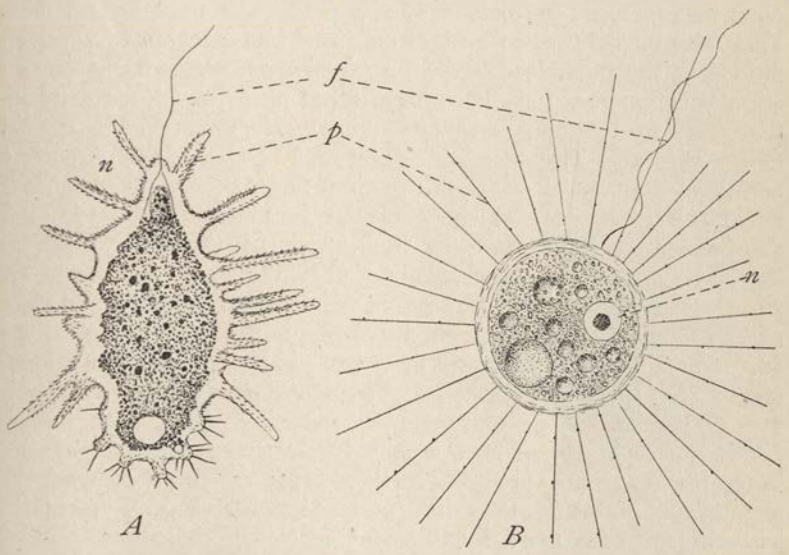

FIG. 137.—Types of Rhizomastigidæ. *A*, *Mastigamœba aspera*. *B*, *Actinomonas mirabilis; f*, flagella; *p*, pseudopodia. (From Calkins after F. E. Schultze and Sav. Kent.)

Reproduction is by longitudinal division in motile (*Multicilia*) or in resting phases (*Mastigamœba setosa*).

The individuals are naked and free-swimming or amœboid and creeping, save when attached by stalks as in *Pteridomonas* or *Actinomonas* (Fig. 137). Pseudopodia are varied; sometimes ray-like or branching (*Pteridomonas*), sometimes lobose (*Mastigamœba, Mastigella*) sometimes axopodia (*Dimorpha*). The flagellum is simple as a rule but the swimming flagellum may be accompanied by one or two secondary flagella (*Pteridomonas*) or by a trailing flagellum (*Cercobodo, Bodopsis*) or the latter may pass through the

substance of the cell as a rhizoplast thus forming an axial fibril (*Cercomastix*). The method of insertion is quite variable (see Chapter II). Fertilization processes, involving macrogametes and microgametes have been described in detail by Goldschmidt (1905), but there is considerable scepticism over his results.

Order II. **PROTOMASTIGIDA.**

The flagellates included in this Order are little known for the most part, and their affinities are obscure. They are amongst the smallest of the Protozoa and are abundant in waters everywhere, particularly in stagnant pools and infusions. Here also are included some of the most pernicious of the protozoan parasites of man. They well illustrate the power of continued adaptation to new conditions in the host, leading to progressive parasitism whereby an original commensal may become a lethal parasite. Such a transition is shown by the genera *Leptomonas, Herpetomonas, Crithidia, Leishmania* and *Trypanosoma*. The first of these is apparently a harmless commensal of the intestine (*Leptomonas* of nematode worms, *Herpetomonas* of the common house-fly) with quiescent, non-flagellated stages free in the lumen of the gut. *Crithidia* species live in the intestinal tracts of various larval forms of insects and are remarkable in having a free-swimming or nectomonad stage and a quiescent usually attached, resting, haptomonad stage. The nectomonads become transformed into haptomonads by attachment to the surface of epithelial cells, where they lose their flagella, live and multiply as extracellular parasites (Fig. 138). With *Leishmania*, parasitism goes a step further; the nectomonads are in the blood and are carried to various organs of the body where as intracellular parasites they live and multiply. Various forms of leishmaniasis are caused by different species of this genus. Kala-azar of India is due to *L. donovani*, Mesnil; tropical ulcer of the near East to *L. tropica*; infantile ulcer of the Mediterranean regions to *L. infantum* (Nicolle); and the Brazilian disease known as Espundia to *L. tropica* var. *Americana* or *Braziliensis* (Vianna). In all of these diseases the quiescent phases are passed in cells of different organs. *Trypanosoma lewisi* and *T. cruzi*, the former a rat parasite, the latter a human parasite and cause of American trypanosomiasis (Chagas' disease) have similar intracellular stages, the latter in the mammalian host, the former in the intestinal cells of the transmitting host a rat-flea *Ceratophyllus fasciatus* (Fig. 116, p. 238).

The trypanosomes of African sleeping sickness (*T. gambiense* and *T. rhodesiense*) affect the human host in quite a different way. This is a disease of the lymphatics and the characteristic symptoms are

due to atrophy of the brain cells through lack of nutrition brought about by collections of parasites and lymphocytes (Bruce).

The Order Protomastigida includes a great variety of heterogeneous types of flagellates and is rather a catch-all of promiscuous forms than a homogeneous and clearly defined group of Protozoa. Flagella, for the most part, are limited in number to one or two, but three are present in the bilaterally symmetrical Trimastigidæ. If two are present they may be equal in length (Amphimonadidæ) in which case no distinction can be made between primary and secon-

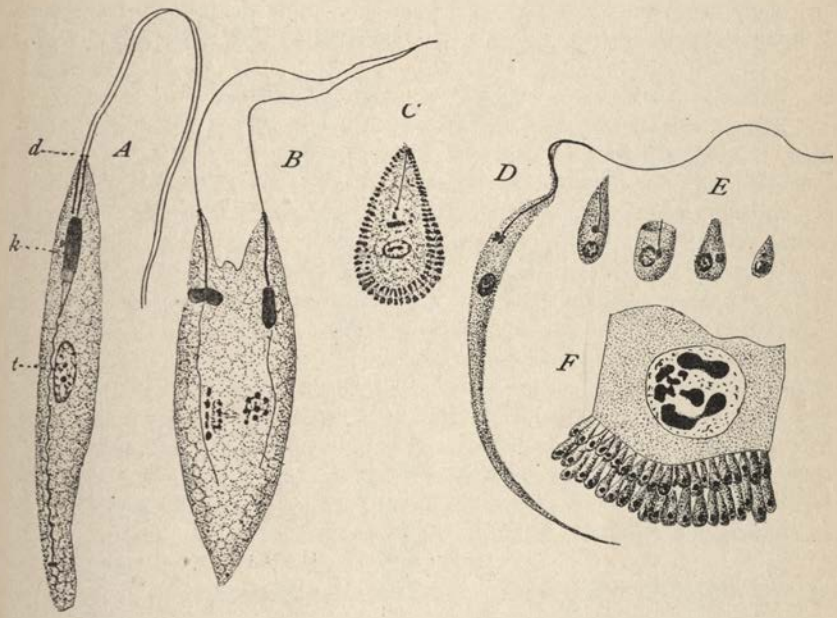

FIG. 138.—Protomonads. *A*, *B*, *Herpetomonas musca-domesticæ; C*, resting stage of same; *D*, *Crithidia subulata*, nectomonad; *E*, resting forms of same; *F*, haptomonads of same attached to epithelial cell; (*d*) basal bodies; (*k*) parabasal body; (*t*) nucleus. (From Calkins after Prowazek and Leger.)

dary flagella; or, there may be a distinct difference between primary and secondary (Monadidæ, Bodonidæ). The secondary flagellum may be directed forward (*Monas*), laterally (*Prowazekia*) or backward as a trailing flagellum (*Bodonidæ*) or may be attached to the periplast to form an undulating membrane (*Cryptobiidæ*). The single flagellum may arise from the anterior part of the cell (Bicœcidæ, Oicomonadidæ, Choanoflagellidæ and Phalansteriidæ) or with an undulating membrane from the posterior end (Trypanosomatidæ). Protoplasmic collars surrounding the bases of the flagella like the collar cells of sponges, are present in Choano-

flagellidæ and Phalansteriidæ. Colony formation is frequent, the spheroid, arboroid and catenoid colonies, however, never attain to the complexity and differentiation of the phytomastigote colonies.

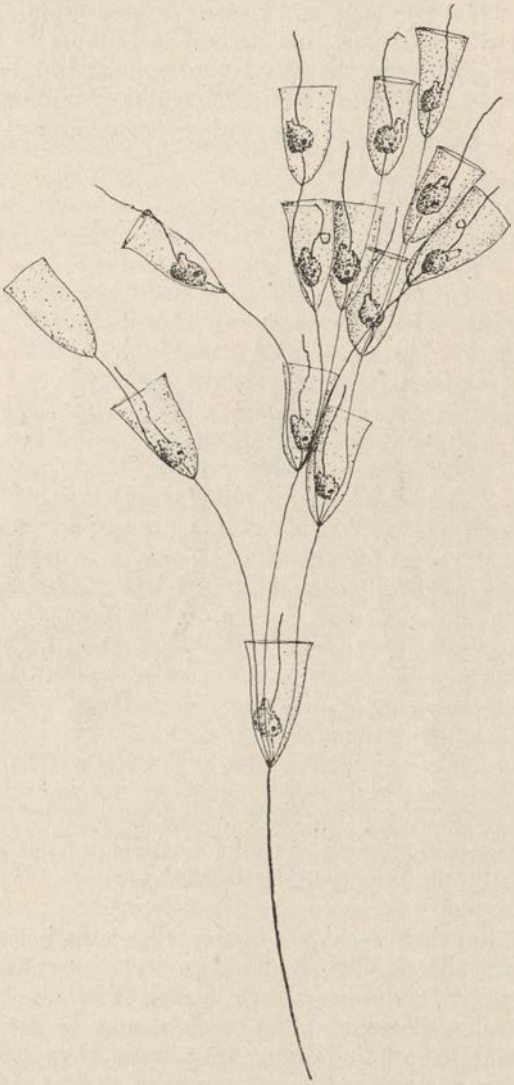

FIG. 139.—Arboroid colony of protomonads, *Poteriodendron petiolatum*. (Original.)

Somatella formation is frequent in parasitic forms or this may be replaced by multiple division within a cyst. Sexual processes have been repeatedly described but the observations are not convincing

and lack confirmation. The same is true of the autogamous processes described for *Anthophysa, Prowazekella, Bodo* and *Leptomonas.*

Family 1. **Trimastigidæ,** Pascher and Lemmermann.—Bilaterally symmetrical forms with one primary and two trailing flagella. Two genera are included neither of which is adequately described—*Dallingeria,* Kent, and *Macromastix,* Stokes.

Family 2. **Bicœcidæ.**—House-dwelling forms with rudimentary collar, single or colonial (Fig. 139); with (*Histiona*) or without (*Bicosœca*) contractile stalk.

Family 3. **Oicomonadidæ.**—Uniflagellate small forms of oval or elongate shape, frequently amœboid. The flagellum is anchored in the body by a simple basal body or by a rhizoplast with basal body.

Family 4. **Trypanosomatidæ.** — From a practical medical or public hygiene point of view, the Trypanosomatidæ are among the most important of all Protozoa. Several known diseases of man and of domesticated animals are due to them, and other diseases are possible through adaptations of forms which are now commensals or parasitic in lower types of animals. They differ from other Protomastigida in the possession of a well-marked periplast which gives a definite form to the body but still allows plasticity. The form is usually ellipsoidal and pointed at one or at both ends (Fig. 48, p. 97). Mouth parts are absent and nutrition is osmotic. The flagellum is single arising from a basal body which is either independent of a blepharoplast or united with it, and follows the margin of an undulating membrane to the anterior end of the body where it becomes a free whip. A parabasal body is present in some cases. In *Trypanosoma lewisi* the developmental cycle in the arthropod intermediate host has been fully worked out by Minchin and Thompson (1915, see Fig. 118, p. 238).

Family 5. **Choanoflagellidæ,** Stein.—The collared flagellates are small forms (10 μ to 20 μ) with a single flagellum and with an occasional second flagellum which is used for anchoring. The essential characteristic is the possession of a delicate protoplasmic collar, sometimes double, in the form of a funnel surrounding the flagellum (Fig. 18, p. 39). This may be rudimentary in some forms which are here included in the Bicœcidæ. They are sedentary forms which, if temporarily freed, swim with the flagellum backward. Contractile vacuoles are simple and one or two in number. Nutrition is holozoic or saprozoic, and reproduction is by simple division with frequent colony formation.

Family 6. **Phalansteriidæ.**—These are small forms, also provided with collars, but both collars and cell bodies are embedded in jelly, the flagella alone protruding. Only one genus—*Phalansterium* (Fig. 20, p. 41).

Family 7. **Cryptobiidæ**, Leidy.—Highly metabolic parasitic forms with primary, free, flagellum and with undulating membrane ending in a terminal posterior whip. Two genera: *Cryptobia* (*Trypanoplasma*), chiefly fish blood parasites, and *Trypanophis*, a parasite of Siphonophora.

Family 8. **Amphimonadidæ**, Doflein.—Forms with two equal flagella, naked and free-swimming, or in some types living in gelatinous houses or tubes. Colony formation is frequent; contractile vacuoles simple, one or two in number. Reproduction by simple division in the free-swimming state.

Family 9. **Monadidæ**, Stein.—Free-living or attached forms provided with two dissimilar flagella, one of which, secondary, is quite short. Contractile vacuoles simple and one or two in number. Colony formation frequent. Some species of *Monas* and of *Anthophysa* form cysts which are similar to those of Chrysomonadida in having neck-like processes closed by plugs (Fig. 5, p. 24); silica, however, has not been detected in the cyst walls.

Family 10. **Bodonidæ**, Bütschli.—Monads with two flagella one of which (primary) is directed forward, the other (secondary) drags behind. In many cases both arise from a well-marked snout-like process (*Bodo*) which, in *Rhynchomonas*, is drawn out into a tentacle replacing the primary flagellum. In *Dinomonas* both flagella are directed forward and are of almost equal length. The kinetic apparatus is complex; contractile vacuoles simple, often numerous. Nutrition is holozoic and reproduction is by simple division. Sexual processes have been described but without satisfactory evidence.

Order III. **POLYMASTIGIDA**, Blochmann.

The flagellates included in this Order are again a heterogeneous lot and their classification is purely tentative. Many of them are extremely minute and details of structure, particularly of the delicate flagella and their distribution on the body, are easily overlooked or misinterpreted. Synonymy in consequence is very confused. After trying in vain to harmonize the many taxonomic systems which have been advocated I have abandoned the idea of grouping forms in sub-orders and families which connote genetic relationships, and in the following classification have presented the established genera according to their similarities in structure under the non-committal terms of "Tribes" and "Groups."

The structural elements on which the grouping is based are flagella, cytostomes and kinetic elements, the axostyle in particular. Tribes are designated according to the single (Monozoa) or double (Diplozoa) condition of the cytostome and accompanying kinetic elements; or multiple (Polyzoa) according to the presence of many

sets of what are obviously unit aggregates of nuclei and kinetic elements (karyomastigonts and akaryomastigonts of Janicki).

The great majority of forms included here are parasitic and most of them are simple with one cytostome if any (Tribe Monozoa). The kinetic elements are highly developed much more so than in free-living types. In a small number of genera which are usually grouped as the Order Distomatida (Klebs), the cytostome is double and the organisms are bilaterally symmetrical. In the majority of these the mouths are separated and occupy symmetrical positions. In *Giardia*, however, the mouths have come together to form a single suctorial cytostome (Fig. 140). There is some justification for Kofoid's (1920) hypothesis that *Giardia* has resulted from the division of a type something like *Chilomastix* with subsequent re-

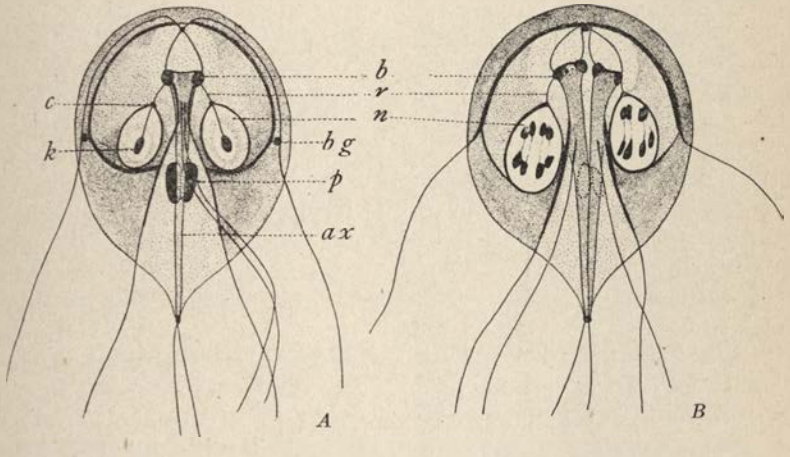

FIG. 140.—*Giardia muris; ax*, axostyle; *b*, blepharoplast; *bg*, basal body; *c*, centriole; *k*, endosome; *n*, nuclei; *p*, parabasal body. (After Kofoid and Christianson.)

fusion as in the formation of a double *Uroleptus mobilis* (see p. 466) or of a double *Glaucoma scintillans* described by Chatton (1921). Like *Giardia* the majority of these forms are binucleated and each side has its equivalent complex of kinetic elements. Kofoid points out that *Chilomastix* is almost an exact duplicate of the right side of *Giardia*, and the various types included here may be interpreted as having arisen in a similar manner from flagellates with originally two, three, or four, flagella. For this reason we have grouped them here under the Tribe Diplozoa.

Somatella formation is a frequent phenomenon in the life history of these parasitic flagellates. Within the cell membrane the nucleus and kinetic elements divide, usually from 2 to 4 times, and 4, 8, or 16, future individuals are thus contained within the mem-

brane of the parent cell (see p. 237). This method of sporulation is frequent in Protomastigida and equally common amongst these Polymastigidæ. As the Diplozoa may represent incompletely divided individuals, so one group of the Polymastigida are generally interpreted as incompletely separated daughter cells which have arisen through somatella formation. With Janicki, Doflein, Koidzumi and others we include all such multiple forms under the Tribe Polyzoa. Each of the one hundred or more nuclei of *Stephanonympha* (Fig. 141) is accompanied by similar kinetic elements, flagella, blepharoplast and rhizoplast and, according to the hypoth-

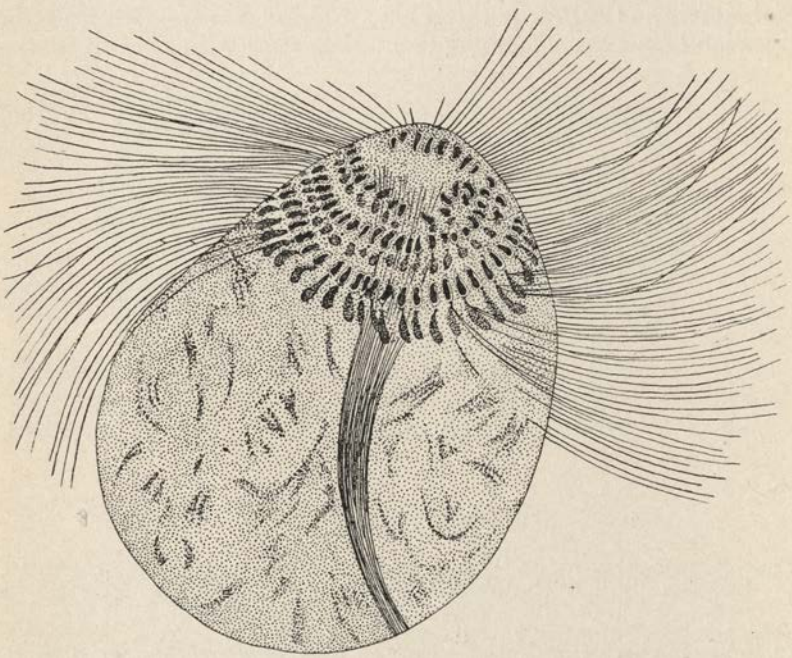

FIG. 141.—*Stephanonympha sylvestri;* with many nuclei, kinetic groups, and flagella. Rhizoplasts unite to form the inner axial strand. (After Janicki.)

esis, each complex represents a single ancestral organism (karyomastigont). In *Calonympha* (Fig. 49, p. 98) the nuclei are less numerous and some sets of kinetic elements are not accompanied by nuclei, such aggregates are called akaryomastigonts by Janicki. In all of these forms the rhizoplasts come together to form a distinct, unified, and sometimes huge, axial strand (Fig. 141). This axial strand is morphologically quite different from the axostyle of monozoic and polyzoic forms, where, according to Kofoid, this element acts as an organ of locomotion, assisting a *Trichomonas* for example, in making its way through the dense mucus of its environment. In

Pyrsonympha and *Dinenympha* (Monozoa) the axostyle may vibrate actively in the endoplasm of the cell. In the majority of forms, however, the axostyle is fairly rigid, projecting in some genera beyond the posterior cell periphery (*Trichomonas, Foaina,* etc.).

Most of the Polymastigida are parasitic and colony formation is unknown. In many genera one at least of the flagella is trailing or united with the periplast to form an undulating membrane. The kinetic elements are complex and present in some way or other all known derivatives of the blepharoplast, including basal bodies, blepharoplast, parabasal body, centrioles and rhizoplasts. Chromosomes of definite form and number are characteristic and a central spindle which in most flagellates is intranuclear (centrodesmose) is here, in the majority of forms at least, outside the nuclear membrane (paradesmose).

The periplast is usually delicate permitting metaboly or plastic changes of the body form. Contractile vacuoles are absent. A mouth opening is common and may be accompanied by a definite pharynx. Nutrition is usually holozoic but some forms are saprozoic. Reproduction is typically by longitudinal division but multiple division with somatella formation is widely distributed. Encystment is practically universal and infection of new hosts is brought about by such cysts through contaminative infection. Sexual processes have been described but the data are unconvincing and the interpretation very questionable. Bunting (1922) describes a species of *Tetramitus* as the flagellated phase of a coprozoic Amœba.

Polymastigida are characteristic parasites of the digestive tract and may usually be found in the intestine of any vertebrate particularly mammals and man, or in the intestine and rectum of many kinds of invertebrates particularly of insects. Their pathogenic effects on the host are questionable but in no case are they as severe as those due to the Protomastigida. *Octomitus salmonis* (Moore, 1922) however, like *Leishmania* and *Trypanosoma* has an intracellular developmental phase whereby multitudes of cells of young trout are destroyed and the trout killed (Davis, 1923).

Order IV. **HYPERMASTIGIDA**, Grassi.

The organisms included in this Order are parasites of insects, particularly of Termites and are the most highly specialized of the Mastigophora. Many authors indeed, make them a distinct class of the Protozoa. Flagella are numerous and may arise from all parts of the body but they are always connected by coördinating fibrils or rhizoplasts, with a centroblepharoplast at the anterior end.

The peculiar symbiotic relations of Termites and these Hypermastigida have been cleared up by the excellent work of Cleveland

(see p. 193). Food-taking at the posterior end of the body through the activity of pseudopodia-like processes has been described by Swezy (1923), and at the anterior end of the body by a process similar to the method described by Rhumbler for *Amœba*, as invagination (Cleveland, 1925). The food substances are larger or smaller fragments of wood, implying the activity of exceptional amylolytic digestive ferments (cellulase). The products of digestion are glycogens which are used as nutriment by the termites. Termites deprived of these flagellates die, and if the wood diet of the Termites is stopped the flagellates die (Cleveland).

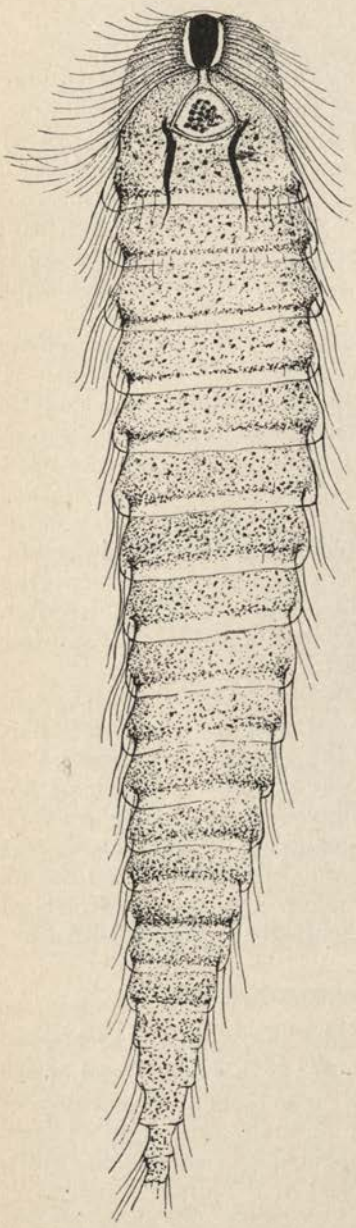

Fig. 142.—*Teratonympha mirabilis*, one of the Hypermastigidæ. (After Koidzumi.)

The general covering of flagella has led to the inclusion of these flagellates with the Infusoria. The organization, however, has nothing in common with that of the Ciliata; they are uninucleate and their kinetic complex is homologous with nothing in the ciliate cell, but is best interpreted as a special development of the flagellate type of kinetic apparatus. This, in a typical case, consists of a conspicuous mass of substance deeply staining with hematoxylin, which forms the center of a radiating system of fibrils (rhizoplasts) running to the cortex in various parts of the cell where they end in basal bodies which give rise to long flagella. The central mass of this system is termed a centroblepharoplast by Kofoid because of its function in cell division (Fig. 51, p. 100). At this time it becomes a huge centrosome which divides to form an amphiaster with central spindle fibers and astral rays formed by the converging rhizoplasts while the cen-

tral spindle fibers form a paradesmose. An axial strand of associated rhizoplasts is also present in many types. Reproduction by longitudinal division is characteristic throughout, the kinetic apparatus in many cases being discarded and new ones formed. Multiple division and somatella formation is also characteristic (Lophomonadidæ).

We follow Grassi (1917) and Koidzumi (1921) in placing the numerous genera of Hypermastigida in six families, two of which, Staurojœnidæ and Teratonymphidæ, have only one genus each. The latter is represented by a most unusual type, *Teratonympha*, Koidz. with plications of the periplast which give it a characteristic segmented appearance (Fig. 142).

Family 1. **Lophomonadidæ**, Grassi.—In these forms the many flagella are concentrated at the anterior end of the cell and arise from a circle of basal bodies and blepharoplasts which form a collar-like aggregate of kinetic elements. In all species thus far described rhizoplasts unite posterior to the nucleus to form an axial strand (Fig. 98, p. 212). A mouth being absent solid food substances are ingested at any part of the posterior end of the body (Janicki). Division processes are complicated and involve the degeneration and disappearance of the old kinetic apparatus and flagella, and the formation of a new complex of nucleus, collar and flagella for each of the daughter cells (Fig. 98). Multiple division into eight occurs during encystment (*Lophomonas*).

Family 2. **Jœnidæ**, Grassi.—As in the preceding family the many flagella are confined to the anterior end or, exceptionally, distributed over the larger part of the body (*Mesojœnia*). Some of the flagella are directed posteriorly covering part of the body as with a mantle (*Jœnia*). A conspicuous and powerful axial strand is invariable. Mode of life and reproduction are the same as in the Lophomonadidæ. They are parasites mainly of *Calotermes* species.

Family 3. **Trichonymphidæ**, Janicki.—Very large parasites of termites with numerous and long flagella arising from the anterior third of the body. The flagellar area is distinctly marked off into an anterior and a posterior zone. The anterior tip is free from flagella and is provided with a knob or tubular structure termed the "head organ." An axial strand is absent but a fluid-filled vacuole is present at the anterior end. The kinetic apparatus is very complex and is re-formed at division.

Family 4. **Holomastigotidæ**, Janicki.—Here the entire body is covered with cilia-like flagella which may be closely set, without especial arrangement, or arranged in spiral rows. A "head organ" and an axial strand may also be present.

III. KEY TO COMMON GENERA OF MASTIGOPHORA.

Flagellates colored by chlorophyll and their colorless relations; vacuoles simple or complex

Class 1. PHYTOMASTIGODA

Flagellates without chlorophyll or relation to chlorophyll-bearing forms . . . . . Class 2. ZOÖMASTIGODA

CLASS I. PHYTOMASTIGODA, DOFLEIN.

1. Without cellulose shell, furrow, or transverse flagellum 2
   With cellulose shell, transverse flagellum and furrow . . . . . Order 3. DINOFLAGELLIDA
2. Chlorophyll yellow or brown; vacuoles simple 3
   Chlorophyll green; vacuoles simple or complex 4
3. Gullet absent; cells not flattened . . Order 1. CHRYSOMONADIDA
   Gullet present; cells usually flattened
   Order 2. CRYPTOMONADIDA
4. Cellulose membrane; no gullet; vacuole simple . . . . . Order 4. PHYTOMONADIDA
   With gullet; vacuole system complex 5
5. Metabolic products paramylum . . . Order 5. EUGLENIDA
   Metabolic products oil . . . . . Order 6. CHLOROMONADIDA

ORDER I. CHRYSOMONADIDA.

1. Motionless stage dominant or permanent 2
   Motile stage dominant . . . . . Sub-order 1. EUCHRYSOMONADINA
2. No flagella known; rhizopodia forms
   Sub-order 2. RHIZOCHRYSIDINA
   Palmella-stage dominant . . . . . Sub-order 3. CHRYSOCAPSINA

SUB-ORDER 1. Euchrysomonadina.

With one apical flagellum . . . . . Family 1. CHROMULINIDÆ
With two, equal, apical flagella . . . . Family 2. ISOCHRYSIDÆ
With two, unequal, apical flagella . . Family 3. OCHROMONADIDÆ
With calcareous discs and rods . . . . . Family 4. COCCOLITHOPHORIDÆ
With simple or fenestrated skeleton . Family 5. SILICOFLAGELLIDÆ

Family 1. **Chromulinidæ.**

1. Chromatophores indefinite or network
   Tribe 1. CHRYSAPSIDINÆ
   Chromatophores definitely formed 2
2. Test if present, simple, not sculptured
   Tribe 2. EUCHROMULINIDÆ
   Test sculptured; vacuole simple or double
   Tribe 3. MALLOMONADIDÆ

Tribe 1. CHRYSAPSIDINÆ.

One genus with tribal characteristics . . Genus *Chrysapsis*

Tribe 2. EUCHROMULINIDÆ

1. With marginal tentacles; truncate
   Sub-family 1. CRYTOPHORINÆ
   No marginal tentacles 2
2. Naked; without test or house 3
   With test or house 4

3. Not laterally compressed....Sub-family 2. CHROMULININÆ
Cell laterally compressed....Sub-family 3. SPHALEROMANTINÆ
4. Test delicate; only partly filled
Sub-family 4. LEPOCHROMULININÆ
Test thick; spherical; close-fitting
Sub-family 5. KYTOCHROMULININÆ

Sub-family 1. *Cyrtophorinæ*
1. With contractile stalk..... 2
No stalk; cells in anterior third of test
Genus *Palatinella*
2. Tentacles much shorter than the flagellum
Genus *Pedinella*
Tentacles much longer than the flagellum
Genus *Cyrtophora*

Sub-family 2. *Chromulininæ*
Circular in optical cross-section.....Genus *Chromulina*
Pyramidal; triangular in cross-section..Genus *Pyramidochrysis*

Sub-family 3. *Sphaleromantinæ*
With characteristics of the sub-family, one genus.....Genus *Sphaleromantis*

Sub-family 4. *Lepochromulininæ*
1. Free-swimming..... 2
Attached..... 3
2. Test with wide opening.....Genus *Kephyrion*
Test calcareous with pores for fine pseudopodia.....Genus *Porochrysis*
3. Test without "straddling" processes.Genus *Lepochromulina*
Test with two straddling arms; attached to algæ.....Genus *Chrysopyxis*

Sub-family 5. *Kytochromulininæ*
One genus with characters of the sub-family
Genus *Chrysococcus*

Tribe 3. MALLOMONADIDÆ

Family 1. **Mallomonadidæ.**
1. Solitary forms..... 2
Colonial; in jelly; two spines from each cell
Genus *Chrysosphærella*
2. Pellicle close-fitting, soft.....Genus *Microglena*
Pellicle rigid; with silicious plates and needles.....Genus *Mallomonas*

Family 2. **Isochrysidæ.**
Without close-fitting sculptured pellicle
Sub-family 1. ISOCHRYSINÆ
With close-fitting sculptured pellicle
Sub-family 2. EUHYMENOMONADINÆ

Sub-family 1. *Isochrysinæ*
1. Without test; colonial in jelly; free swimming.....Genus *Syncrypta*
With test..... 2
2. Short stalk or none; often with test partition.....Genus *Derepyxis*
Long stalk and test.....Genus *Stylochrysalis*

Sub-family 2. *Euhymenomonadinæ*
1. Solitary forms.....Genus *Hymenomonas*
Colonial forms..... 2
2. Globular colonies; cells radially arranged
Genus *Synura*

Band-form colonies; cells triangular, broad at base ..... Genus *Chlorodesmus*

Family 3. **Ochromonadidæ.**

Cells naked, solitary or colonial. Sub-family 1. OCHROMONADINÆ
With tests, tests often complex. Sub-family 2. LEPOCHROMONADINÆ

Sub-family 1. *Ochromonadinæ*

1. Solitary forms 2
   Colonial forms 3
2. Colorless forms with stigmata ..... Genus *Heterochromonas*
   With chromatophores, usually without stigmata ..... Genus *Ochromonas*
3. Colonies globular or ellipsoidal 4
   Colonies wheel or funnel-shape ..... Genus *Cyclonexis*
4. Cells peripheral, no inner processes, irregular ..... Genus *Uroglenopsis*
   Cells fixed by inner gelatinous, branched, processes ..... Genus *Uroglena*

Sub-family 2. *Lepochromonadinæ*

1. Tests with external markings or processes Genus *Hyalobryon*
   Tests simple, no markings or processes ... 2
2. Free-swimming forms 3
   Attached forms 5
3. Cell at base of cup, no stalk 4
   Stalk of cell attached to base of cup Part of Genus *Dinobryon*
4. Test keg-shape with cross constrictions Genus *Pseudokephyrion*
   Test oval or ellipsoid; no cross constrictions Genus *Kephyriopsis*
5. Tests with long, often delicate, stalks 6
   Tests without stalks; cells with contractile stems ..... Genus *Dinobryon*
6. Tests globular or vase-shape; cells at bottom ..... Genus *Stylopyxis*
   Tests beaker-shape, narrow; cells at mouth Genus *Poteriochromonas*

Family 4. **Coccolithophoridæ.**

Shell plates are unperforated discs (discoliths) ..... Sub-family 1. SYRACOSPHÆRINÆ
Shell plates are perforated (tremaliths) Sub-family 2. COCCOLITHOPHORINÆ

Sub-family 1. *Syracosphærinæ*

1. Discs without spinous floating processes ... 2
   Discs with spinous processes 3
2. Cells with one flagellum ..... Genus *Pontosphæra*
   Cells with two equal flagella ..... Genus *Syracosphæra*
3. Body covered with disc-plates 4
   Body plates absent, long floating processes Genus *Halopappus*
4. Equatorial plates not modified as floats Genus *Michaelsarsia*
   Equatorial plates modified as floats 5
5. Equatorial processes beaker-shape .. Genus *Syracosphæra*
   Floating processes horn-shape ..... Genus *Torosphæra*

Sub-family 2. *Coccolithophorinæ*

1. Perforated discs without floating processes
   Genus *Coccolithophora*
   Perforated discs with floating processes... 2
2. Floating processes club-shape.....Genus *Rhabdosphæra*
   Floating processes with terminal discs
   Genus *Discosphæra*

Family 5. **Silicoflagellidæ.**

1. Silicious skeleton a simple ring.....Genus *Mesocena*
   Basal ring with bars to form hat-like skeleton..... 2
2. Silicious bars one to four in number. Genus *Dictyocha*
   Silicious bars more than four in number... 3
3. Simple apical ring formed by fused bars
   Genus *Distephanus*
   Complex apical ring.....Genus *Cannophilus*

Sub-order 2. **Rhizochrysidina.**

1. Naked forms..... 2
   With tests or houses..... 4
2. Solitary forms.....Genus *Rhizochrysis*
   Colonial forms..... 3
3. Chain-form or band-like colonies...Genus *Chrysidiastrum*
   Wheel-form colonies.....Genus *Chrysostephanosphæra*
4. Tests oval or ellipsoid, stalks long and fine
   Genus *Stylococcus*
   Tests with broad bases; sessile.....Genus *Lagynion*

Sub-order 3. **Chrysocapsina.**

Growth general, not limited to ends of branches.....Family Chrysocapsidæ
Growth limited to ends of branches; apical
Family Hydruridæ

Family 1. **Chrysocapsidæ.**

Palmella aggregate small; not branching
Genus *Chrysocapsa*
Palmella aggregate branched or irregular
Genus *Phæosphæra*

Family 2. **Hydruridæ.**

One genus with characters of family...Genus *Hydrurus*

Order II. **CRYPTOMONADIDA.**

Motile flagellated stage predominant
Sub-order 1. Eucryptomonadina
Palmella-stage predominant.....Sub-order 2. Phæocapsina

Sub-order 1. **Eucryptomonadina.**

Anterior end obliquely truncated; with furrow
Family 1. Cryptomonadidæ
Cells bean-shape; furrow lateral, near equator
Family 2. Nephroselmidæ

Family 1. **Cryptomonadidæ.**

1. Furrow median, not deepened to a gullet.. 2
   Furrow insunk, forming distinct gullet.... 5

2. Chromatophores blue to blue-green..... 3
Chromatophores brown, red, or green..... 4
3. One or two large chromatophores...Genus *Chroömonas*
Many small, discoidal chromatophores
Genus *Cyanomonas*
4. Chromatophores green or brown, rarely reddish.....Genus *Cryptochrysis*
Chromatophores red, usually single.Genus *Rhodomonas*
5. Chromatophores yellow..... 6
Colorless forms; no chromatophores..... 7
6. Free-living forms; two lateral chromatophores.....Genus *Cryptomonas*
Symbiotic; "yellow cells" in part, of Foraminifera.....Genus *Chrysidella*
7. Flagella (two) from center of anterior end; saprozoic.....Genus *Chilomonas*
Flagella at corner of anterior end; holozoic
Genus *Cyathomonas*

Family 2. **Nephroselmidæ.**
Furrow and stigma distinct; no gullet..Genus *Protochrysis*
Furrow indistinct; with gullet; no stigma
Genus *Nephroselmis*

Sub-order 2. **Phæocapsina.**

Gelatinous masses irregular; no threads
Family 1. Phæocapsidæ
Gelatinous masses branched; with long hairs
Family 2. Phæothamnionidæ

Family 1. **Phæocapsidæ.**
One genus with family characters.....Genus *Nægeliella*
Family 2. **Phæothamnionidæ.**
One genus with family characters.....Genus *Phæothamnion*

Order III. **DINOFLAGELLIDA.**

Body naked or shelled; girdle and sulcus at some stage.....Sub-order Diniferina
Naked or with bivalve shell; no sulcus, no girdle.....Sub-order Adinina
Naked; no sulcus or girdle; no transverse flagellum.....Sub-order Cystoflagellina

Sub-order 1. **Diniferina.**

1. Naked or with delicate one-piece shell; girdle and sulcus distinct at some stage
Tribe 1. Gymnodinioidæ
With skeletal elements, plates or membrane 2
2. No enveloping cuirass; skeletal elements embedded or superficial.....Tribe 2. Amphilothioidæ
With theca or cuirass; epitheca and hypotheca separated by girdle plates Tribe 3. Peridinioidæ

Tribe 1. Gymnodinioidæ
1. With delicate one-piece cellulose shell
Genus *Glenodinium*
Body without shell, naked..... 2

2. Girdle and sulcus obscure; flagella thread-like.....Family 1. PROTODINIFERIDÆ
Girdle and sulcus distinct; transverse flagellum ribbon-like..... 3
3. Individuals permanently colonial Family 3. POLYKRIKIDÆ
Individuals not colonial..... 4
4. With ocellus.....Family 5. POUCHETIIDÆ
Without ocellus..... 5
5. With tentacle.....Family 4. NOCTILUCIDÆ
Without tentacle..... 6
6. Not parasitic.....Family 2. GYMNODINIIDÆ
Parasitic.....Family 6. BLASTODINIDÆ

Family 1. **Protodiniferidæ.**

1. Flagellum encircling tentacle-like process.. 2
Girdle short; tentacle not encircled..Genus *Protodinifer*
2. Both flagella wound about conical tentacle Genus *Hemistasia*
Both flagella free.....Genus *Oxyrrhis*

Family 2. **Gymnodiniidæ.**

1. Girdle one-half turn only.....Genus *Hemidinium*
Girdle more than one-half turn..... 2
2. Girdle anterior; epicone relatively minute Genus *Amphidinium*
Girdle median, sub-median, or spirally wound..... 3
3. Girdle sub-median.....Genus *Gymnodinium*
Girdle posterior or spirally wound..... 4
4. Girdle posterior.....Genus *Torodinium*
Girdle spirally wound..... 5
5. Spiral girdle less than one and one-half turns.... Genus *Gyrodinium*
Spiral one and one-half or more turns Genus *Cochlodinium*

Family 3. **Polykrikidæ.**

One genus with characters of family...Genus *Polykrikos*

Family 4. **Noctilucidæ.**

Gymnodinium-like girdle persistent....Genus *Pavillardia*
Girdle in swarm-spore stage; adult with tentacle.....Genus *Noctiluca*

Family 5. **Pouchetiidæ.**

1. Girdle as in Gymnodinium, no displacement.....Genus *Protopsis*
Girdle spirally wound..... 2
2. With nematocysts.....Genus *Nematodinium*
Without nematocysts..... 3
3. Without posterior tentacle.. Genus *Pouchetia*
With posterior tentacle..... 4
4. Epicone and hypocone nearly equal.Genus *Proterythropsis*
Epicone smaller than hypocone.....Genus *Erythropsis*

Family 6. **Blastodinidæ.**

1. Ectoparasitic forms on eggs or appendages 2
Endoparasitic forms in gut or body cavity 7
2. Parasites on appendages of pelagic animals 3
Parasites on eggs or in other protozoa.... 5
3. Hemispheres sub-equal..... 4
Anterior hemisphere more developed than posterior.....Genus *Oödinium*

4. Attaching peduncle prolonged in rhizoids
Genus *Apodinium*
Attaching peduncle not prolonged in rhizoids.....Genus *Parapodinium*
5. Parasites on copepod eggs..... 6
Parasites on diatoms.....Genus *Paulsenella*
6. Dinospores Gymnodinium-like.....Genus *Chytriodinium*
Dinospores Spirodinium-like.....Genus *Trypanodinium*
7. Intestinal parasites..... 8
Cœlomic parasites.....Genus *Syndinium*
8. Vegetative forms not attached..... 9
Vegetative forms attached.....Genus *Haplozoön*
9. Products of division independent and separate.....Genus *Schizodinium*
Products of division remain together
Genus *Blastodinium*

Tribe 2. Amphilothiodæ

Four doubtful genera of Dinoflagellida—*Amphilophus* Schütt, *Gymnaster* Schütt, *Achradina* Lohmann, *Monaster* Schütt.

Tribe 3. Peridinioidæ

1. Girdle and sulcus present..... 2
Girdle and sulcus absent, plant-like
Family 3. Phytodinidæ
2. Cuirass usually divided by sagittal suture
Family 2. Dinophysidæ
Shell of distinct plates, no suture. Family 1. Peridinidæ

Family 1. **Peridinidæ.** (Deep sea forms omitted).

1. Cuirass prolonged into horn-like processes 2
Cuirass without horns..... 5
2. Short horns on hypotheca..... 3
Horns on hypotheca and epitheca..... 4
3. Horns two or three in number.....Genus *Peridinium*
Horns more than three in number..Genus *Ceratocorys*
4. One anterior, one to three posterior horns
Genus *Ceratium*
Horns numerous on both halves....Genus *Acanthodinium*
5. Body polyhedral.....Genus *Goniodoma*
Body spheroidal .....Genus *Gonyaulax*

Family 2. **Dinophysidæ.** (Deep sea forms omitted.)

1. Form elongate or needle-like Genus *Amphisolenia*
Form ellipsoidal, ovoidal or spheroidal.... 2
2. Girdle ridges broad, often funnel-like
Genus *Dinophysis*
Girdle ridges narrow, never funnel-like
Genus *Phalacroma*

Family 3. **Phytodinidæ.**

1. Individuals stalked (usually fresh water)
Genus *Stylodinium*
Individuals not stalked..... 2
2. Chromatophores distributed, protoplasm radial.....Genus *Pyrocystis*
Chromatophores peripheral; plasm not radial Genus *Phytodinium*

Sub-order 2. **Adinina.**

With anterior spine-like process.....Genus *Prococentrum*
Anterior end rounded.....Genus *Exuviælla*

Sub-order 3. **Cystoflagellina.**

Two doubtful genera of Dinoflagellates—
Form discoidal with minute flagellum..Genus *Leptodiscus*
Form medusa-like; with velum.....Genus *Craspedotella*

Order IV. **PHYTOMONADIDA.**

1. Four flagella; no firm membrane.Family 1. Polyblepharidæ
   Usually two flagella; with cellulose membranes..... 2
2. Cellulose membrane a single piece..... 3
   Membrane in two valves.....Family 2. Phacotidæ
3. With chromatophores..... 4
   Colorless forms.....Family 3. Polytomidæ
4. Solitary or colonial; flagella two or four
   Family 4. Chlamydomonadidæ
   Colony forms; two flagella.....Family 5. Volvocidæ

Family 1. **Polyblepharidæ.**

1. With green chromatophores.....Genus *Pyramimonas*
   Colorless forms.....Genus *Polytomella*

Family 2. **Phacotidæ.**

1. Bivalve shell distinct at all times...Genus *Phacotus*
   Bivalve condition shown only during division..... 2
2. Shell with lateral ridges.....Genus *Pteromonas*
   Shell without lateral ridges.....Genus *Coccomonas*

Family 3. **Polytomidæ.**

With rounded anterior end; two flagella
Genus *Polytoma*
With obliquely truncated anterior end.Genus *Parapolytoma*

Family 4. **Chlamydomonadidæ.**

1. With four flagella..... 2
   With two flagella..... 3
2. Individuals solitary.....Genus *Carteria*
   Colonial forms.....Genus *Spondylomorum*
3. Cellulose membrane tight fitting..... 4
   Membrane separated by space from cell... 6
4. Cells elongate, spindle-form.....Genus *Chlorogonium*
   Cells spherical or ellipsoidal..... 5
   Cells cuboidal or lobate..... 7
5. One large, cup-shape chromatophore
   Genus *Chlamydomonas*
   Chromatophore band-form about periphery
   Genus *Mesostigma*
6. Flagella pass through jelly of shell..Genus *Sphærella*
   Flagella from protoplasmic strand at periphery.....Genus *Hæmatococcus*
7. Lobate arm-like processes.....Genus *Brachiomonas*
   Variable mound-like excrescences on cell
   Genus *Lobomonas*

Family 5. **Volvocidæ.**

1. Colony flat; cells in one plane..... 2
   Cells united in spheroidal colonies..... 3
2. Flagella directed from one surface only
   Genus *Gonium*

Flagella directed alternately from both sides.....Genus *Platydorina*
3. Cells in meridional plane only.....Genus *Stephanosphæra*
Cells distributed on or in a gelatinous matrix..... 4
4. Colonies of sixteen closely united cells Genus *Pandorina*
More than sixteen cells, not closely associated..... 5
5. Individuals do not form a superficial layer 6
Individuals form a superficial layer of cells Genus *Volvox*
6. Cells not differentiated, thirty-two in number.....Genus *Eudorina*
Cells different in size, somatic and germinal Genus *Pleodorina*

Order V. **EUGLENIDA.**

1. With chromatophores and, usually, stigmata Family 1. Euglenidæ
Without chromatophores..... 2
2. Without stigmata..... 3
With stigmata..... 5
3. With one flagellum..... 4
With two flagella, one directed posteriorly Family 3. Heteronemidæ
4. Single flagellum directed anteriorly Family 2. Astasiidæ
Single flagellum directed posteriorly Genus *Clautriavia*
5. One species of genus Astasia.....Species *A. ocellata*

Family 1. **Euglenidæ.**

1. Flagellum single, form rigid or metabolic.. 2
Two flagella, body metabolic.....Genus *Eutreptia*
Three flagella; commensal in tadpole intestine.....Genus *Euglenomorpha*
2. Solitary, without gelatinous stalk..... 3
Stalked and colonial in resting phase Genus *Colacium*
3. Test or shell absent..... 4
Test or shell present..... 6
4. Rigid or metabolic; paramylum varied Genus *Euglena*
Rigid, never metabolic..... 5
5. Cylindrical; lateral paramylum bodies ring-form.....Genus *Lepocinclis*
Flattened; paramylum body usually single, central.....Genus *Phacus*
6. Test simple or spinous..... 7
Test thick; two band-formed chromatophores.....Genus *Cryptoglena*
7. Test rigid; spines frequent; free-swimming Genus *Trachelomonas*
Test flexible; attached at base.....Genus *Ascoglena*

Family 2. **Astasiidæ.**

1. Cells distinctly metabolic..... 2
Cells rigid..... 6

2. Parabasal body invisible in life..... 3
   Parabasal body visible..... 4
3. Mouth-like fold at base of flagellum Genus *Euglenopsis*
   Without fold at base of flagellum...Genus *Astasia*
4. Spindle-shape; anterior end narrowed..... 5
   Flask-shape, anterior end funnel-like
   Genus *Urceolus*
5. Parabasal body single.....Genus *Peranema*
   Parabasal double or triple.....Genus *Jenningsia*
6. Organisms free-living..... 7
   Organisms parasitic or coprozoic....Genus *Scytomonas*
7. Body crescentic or S-shape.....Genus *Menoidium*
   Body not curved, usually with keels Genus *Petalomonas*

Family 3. **Heteronemidæ.**

1. Cells metabolic..... 2
   Cells rigid..... 4
2. Parabasal body invisible; no cortical granules..... 3
   Parabasal visible; spirally placed cortical granules.....Genus *Dinema*
3. Second flagellum shorter than half the body
   Genus *Distigma*
   Second flagellum as long as, or longer than body.....Genus *Heteronema*
4. Periblast smooth; without keels..... 5
   Periplast with keels..... 8
5. Second flagellum trails behind..... 6
   Second flagellum carried on side....Genus *Metanema*
6. With internal tube.....Genus *Entosiphon*
   Without internal tube..... 7
7. With slit-like ventral furrow.....Genus *Anisonema*
   With broad, pocket-like ventral furrow
   Genus *Marsupiogaster*
8. Body broadly truncate; pyramidal..Genus *Notosolenus*
   Body ellipsoidal..... 9
9. Second flagellum half body length or shorter..... 10
   Second flagellum as long as body or longer
   Genus *Plœotia*
10. Keels inconspicuous, two to four in number
   Genus *Sphenomonas*
   Keels conspicuous; eight in number.Genus *Tropidoscyphus*

Order VI. **CHLOROMONADIDA.**

1. Cells with discoidal, bright green chromatophores..... 2
   Colorless; pseudopodia from ventral surface
   Genus *Thaumatomastix*
2. Cortex without refractile, trichocyst-like rods..... 3
   Cortex with trichocyst-like rods....Genus *Gonyostomum*
3. Cells metabolic; narrowed anteriorly Genus *Vacuolaria*
   Cells narrowed posteriorly; not metabolic
   Genus *Trentonia*

Class II. ZOÖMASTIGODA.

1. With flagella and pseudopodia....Order 1. Pantastomatida
   No pseudopodia; with or without mouth.. 2
2. With one or two primary flagella. Order 2. Protomastigida
   With more than two flagella..... 4
3. Not more than eight flagella except in Calonymphidæ.....Order 3. Polymastigida
   More than eight flagella.....Order 4. Hypermastigida

Order I. PANTASTOMATIDA.

Cells polyaxonic; with many flagella Family 1. Holomastigidæ
Cells monaxonic; one to three, rarely four, flagella.....Family 2. Rhizomastigidæ

Family 1. **Holomastigidæ.**

One genus with the characters of the family
Genus *Multicilia*

Family 2. **Rhizomastigidæ.**

1. Flagellum base with ring of pseudopodia
   Genus *Pteridomonas*
   Flagellum base without pseudopodia..... 2
2. Individuals with stalk; pseudopodia ray-like. Genus *Actinomonas*
   Individuals without stalks..... 3
3. Pseudopodia if present, without axial filaments..... 4
   Pseudopodia with axial filaments...Genus *Dimorpha*
4. Usually one swimming flagellum..... 5
   One swimming, one trailing flagellum..... 7
5. Cytoplasmic axial rod absent..... 6
   Cytoplasmic axial rod present.....Genus *Cercomastix*
6. Flagellum rises from the nucleus....Genus *Mastigamœba*
   Flagellum rises independently of the nucleus
   Genus *Mastigella*
7. Anterior end with trough-like depression
   Genus *Bodopsis*
   Anterior end without depression....Genus *Cercobodo*

Order II. PROTOMASTIGIDA.

1. One flagellum only..... 2
   Two flagella..... 6
   Three flagella; one swimming, two trailing
   Family 1. Trimastigidæ
2. Protoplasmic collar absent..... 3
   Protoplasmic collar present..... 5
3. Tentacle-like process absent..... 4
   Tentacle-like process present...Family 2. Bicœcidæ
4. Undulating membrane absent...Family 3. Oicomonadidæ
   Undulating membrane present; internal parasites.....Family 4. Trypanosomatidæ
5. Collar never enclosed in jelly....Family 5. Choanoflagellidæ
   Collar entirely enclosed in jelly..Family 6. Phalansteriidæ
6. Undulating membrane present; parasites
   Family 7. Cryptobiidæ
   Undulating membrane absent..... 7

7. Flagella of dissimilar length..... 8
   Flagella of similar length.....Family 8. AMPHIMONADIDÆ
8. One primary flagellum, one secondary
   Family 9. MONADIDÆ
   One primary flagellum, one trailing
   Family 10. BODONIDÆ

Family 1. **Trimastigidæ.**
   Trailing flagella arise from anterior tip..Genus *Macromastix*
   Trailing flagella arise below anterior tip
   Genus *Dallingeria*

Family 2. **Bicœcidæ.**
1. Peristomial region thin, skin-like..... 2
   Peristomial region thick, proboscis-like
   Genus *Poteriodendron*
2. Individuals attached by contractile stalk
   Genus *Bicœca*
   Individuals without contractile stalk Genus *Histiona*

Family 3. **Oicomonadidæ.**
1. One swimming flagellum..... 2
   One trailing flagellum.....Genus *Ancyromonas*
2. No cytostome, free-living.....Genus *Oicomonas*
   With cytostome, free-living or parasitic... 3
3. Free-living; mouth small with gullet Genus *Thylacomonas*
   Parasitic in digestive tract..... 4
4. With parabasal body.....Genus *Leptomonas*
   Without parabasal body..... 5
5. Lumen-dwelling; no intracellular stage
   Genus *Rhizomastix*
   With intracellular- non-flagellated stage
   Genus *Leishmania*

Family 4. **Trypanosomatidæ.**
   Undulating membrane extends down the body
   Genus *Trypanosoma*
   Undulating membrane at base of flagellum only.....Genus *Crithidia*

Family 5. **Choanoflagellidæ.**
1. With one protoplasmic collar..... 2
   With two protoplasmic collars (?)..... 10
2. Without test or shell..... 3
   With test or shell..... 9
3. Individuals not embedded in jelly..... 4
   All but collar enclosed in jelly; colonial... 8
4. Solitary; short stalked or sessile....Genus *Monosiga*
   Branched or otherwise colonial; not sessile 5
5. Stalks attached; individuals clustered at ends..... 6
   Individuals in free-swimming colonies.... 7
6. Stalk not branched.....Genus *Codonosiga*
   Stalk branched.....Genus *Codonocladium*
7. Colonies stellate.....Genus *Astrosiga*
   Colonies band-form, simple or branched
   Genus *Desmarella*
8. Individuals without stalks; irregularly placed.....Genus *Proterospongia*
   Individuals with stalk, radially arranged
   Genus *Sphæroica*

9. Cells attached ..... Genus *Salpingœca*
Cells free-swimming ..... Genus *Lagenœca*
10. Individuals without test 11
Individuals with delicate test ..... Genus *Diplosigopsis*
11. Individuals sessile or with short stalk
Genus *Diplosiga*
Individuals with very long stalk .... Genus *Codonosigopsis*

Family 6. **Phalansteriidæ.**
One genus only ..... Genus *Phalansterium*

Family 7. **Cryptobiidæ.**
Without skeletal bars ..... Genus *Cryptobia*
With skeletal bars ..... Genus *Trypanophis*

Family 8. **Amphimonadidæ.**
1 Individual without tubes, tests, or jelly ... 2
Individuals in tubes, tests or jelly 4
2. Cells without keels or ridges 3
Cells with lateral keels ..... Genus *Streptomonas*
3. Cells ovoidal, spindle-shape or spherical
Genus *Amphimonas*
Cells horse-shoe-shape or spiral 5
4. Cells in stalked test ..... Genus *Diplomita*
Cells in gelatinous masses or in tubes 6
5. Cells horse-shoe-shape ..... Genus *Purcilla*
Cells spirally twisted ..... Genus *Spiromonas*
6. Cells in gelatinous masses ..... Genus *Spongomonas*
Cells in gelatinous tubes 7
7. Tubes not united laterally ..... Genus *Cladomonas*
Tubes fused lengthwise (organ-pipe forms)
Genus *Rhipidodendron*

Family 9. **Monadidæ.**
1. Individuals solitary 2
Individuals colonial 5
2. Without tests 3
With tests ..... Genus *Stokesiella*
3. Surface smooth 4
Surface covered with slime and radial threads ..... Genus *Physomonas*
4. Both flagella active ..... Genus *Monas*
Primary flagellum rigid ..... Genus *Sterromonas*
5. Individuals with test ..... Genus *Stylobryon*
Individuals without test 6
6. Cells solitary at ends of branched stalks
Genus *Dendromonas*
Cells colonial at ends of branched stalks .. 7
7. Stalks colorless, rigid ..... Genus *Cephalothamnium*
Stalks yellow or brown; plastic ..... Genus *Anthophysa*

Family 10. **Bodonidæ.**
1. Two unequal swimming flagella .... Genus *Dinomonas*
One swimming flagellum 2
2. One swimming, one trailing, or feeding, flagellum 3
Main flagellum replaced by movable proboscis ..... Genus *Rhynchomonas*
3. Parasitic; parabasal body present ... Genus *Prowazekella*
Parabasal body absent 4

4. Intestinal parasites..... 5
   Free-living forms..... 6
5. With large cytostome.....Genus *Embadomonas*
   No cytostome; with "blastocystis" stage
   Genus *Schizobodo*
6. With deep, gullet-like mouth.....Genus *Phyllomitus*
   Without gullet-like mouth..... 7
7. Broad ventral furrow with rolled edges
   Genus *Colponema*
   Without ventral furrow..... 8
8. Trailing flagellum from anterior end Genus *Bodo* (*Prowazekia*)
   Trailing flagellum from center; feeding dorsal.....Genus *Pleuromonas*
   Posterior flagellum leaves body from end
   Genus *Cercomonas*

Order III. **POLYMASTIGIDA.**

With one cytostome and one kinetic complex
Tribe 1. Monozoa
With two cytostomes and kinetic elements (distomata).....Tribe 2. Diplozoa
Compound individuals (possibly permanent somatellæ).....Tribe 3. Polyzoa

Tribe I. Monozoa

Without cytostome, undulating membrane, or axostyle..... *Group 1*
Without cytostome, without undulating membrane, with axostyle..... *Group 2*
With cytostome; with undulating membrane; without axostyle..... *Group 3*
With cytostome, with undulating membrane, with axostyle..... *Group 4*
With cytostome, without undulating membrane, with axostyle..... *Group 5*

Group 1

1. With one trailing, and two anterior flagella 2
   With more than two anterior flagella..... 3
2. Trailing flagellum leaves body at posterior end.....Genus *Enteromonas*
   Trailing flagellum leaves body half way down.....Genus *Diplocercomonas*
3. With three anterior flagella.....Genus *Tricercomonas*
   With more than three anterior flagella.... 4
4. With four anterior flagella..... 5
   With six anterior flagella, no trailing flagellum.....Genus *Streblomastix*
5. Four equal anterior flagella directed forward 6
   Two equal flagella directed anteriorly, two posteriorly.....Genus *Monocercomonas*
6. Usually free-living, body ellipsoidal or truncated.....Genus *Tetramitus*
   Coprozoic, body pyramidal or triangular
   Genus *Copromastix*

Group 2

1. Axostyle vibrates in endoplasm; four to eight posterior flagella 2
   Axostyle rigid; three anterior, one trailing flagellum 3
   Axostyle rigid, protruding; four anterior flagella . . . . . Genus *Trichomastix*
2. Axostyle attached at posterior end . . Genus *Pyrsonympha*
   Axostyle free at posterior region . . . Genus *Dinenympha*
3. Axostyle does not project; flagella central, apical . . . . . Genus *Devescovina*
   Axostyle projects; flagella apical, not central . . . . . Genus *Foaina*

Group 3

With three anterior flagella all equal . . Genus *Chilomastix*
With four equal, anterior flagella . . . . . Genus *Tetrachilomastix*

Group 4

1. Undulating membrane in cytostome; three anterior flagella . . . . . Genus *Cyathomastix*
   Undulating membrane on body margin . . . 2
2. Two free, anterior flagella . . . . . Genus *Ditrichomonas*
   More than two anterior free flagella 3
3. Three free, anterior flagella . . . . . Genus *Tritrichomonas*
   More than three anterior flagella 4
4. Four free, anterior flagella . . . . . Genus *Trichomonas*
   Five free, anterior flagella . . . . . Genus *Pentatrichomonas*

Group 5

1. With trailing flagellum . . . . . Genus *Eutrichomastix*
   Without trailing flagellum 2
2. With three anterior flagella . . . . . Genus *Protrichomonas*
   With more than three anterior flagella 3
3. With four anterior flagella . . . . . Genus *Polymastix*
   With six anterior flagella . . . . . Genus *Hexamastix*

Tribe II. Diplozoa. With two mouths and sets of kinetic elements.

1. Cytostomes united to form one . . . . . Genus *Giardia*
   Cytostomes separated 2
2. Trailing flagella absent 3
   Trailing flagella present 5
3. Four flagella . . . . . Genus *Gyromonas*
   More than four flagella 4
4. Six flagella . . . . . Genus *Trigonomonas*
   Eight flagella . . . . . Genus *Trepomonas*
5. Food taken only at posterior end 6
   Food taking general; no mouth . . . . . Genus *Octomitus*
6. Posterior end with two lateral mouth grooves . . . . . Genus *Trigonomonas*
   Posterior end with bilabiate, movable snout[1] . . . . . Genus *Urophagus*

Tribe III. Polyzoa. Multiple nuclei and kinetic elements (polymastigonts)

1. Nuclei symmetrically placed, each with kinetic complex 2
   Nuclei less numerous than kinetic complexes . . . . . Genus *Calonympha*
2. Nuclei in a single stratum . . . . . Genus *Stephanonympha*
   Nuclei in two strata; huge axial strand Genus *Diplonympha*

Order IV. **HYPERMASTIGIDA.**

1. Organisms with plications or folds; segmented appearance.....Family 6. Teretonymphidæ
   Organisms without plications or folds..... 2
2. Flagella placed in spiral rows...Family 5. Holomastigotidæ
   Flagella not in spiral rows..... 3
3. Flagella in one or more bundles or tufts... 4
   Flagella not in bundles.....Family 4. Trichonymphidæ
4. One anterior bundle or tuft of flagella.... 5
   Four anterior bundles of flagella.Family 3. Staurojœnidæ
5. Flagella directed anteriorly; parabasal in calyx.....Family 1. Lophomonadidæ
   Some flagella directed posteriorly; parabasal not in calyx.....Family 2. Jœnidæ

Family 1. **Lophomonadidæ.**

With from five to fifteen flagella.....Genus *Eulophomonas*
With more than fifteen flagella.....Genus *Lophomonas*

Family 2. **Jœnidæ.**

1. Without trailing flagellum..... 2
   With one trailing flagellum.....Genus *Parajœnia*
2. Parabasal body "feathered"..... 3
   Parabasal not feathered..... 4
3. Without anterior ridges and striations.... 5
   With anterior ridges and striations.Genus *Jœnopsis*
4. Parabasal a simple curved rod on left of nucleus.....Genus *Jœnina*
   Parabasal a lobed collar below nuclear basket.....Genus *Jœnia*
5. Anterior end of body flat and dense.Genus *Microjœnia*
   Anterior end curved; without dense plate Genus *Mesojœnia*

Family 3. **Staurojœnidæ.**

One genus with characters of the family Genus *Staurojœnia*

Family 4. **Trichonymphidæ.**

1. Flagella arranged in two or more zones... 2
   Flagella in one, anterior, zone..... 3
2. Flagella in three zones.....Genus *Trichonympha*
   Flagella in two zones.....Genus *Pseudotrichonympha*
3. Flagella as long as or longer than body Genus *Leidyonella*
   Flagella relatively short (*Gymnonympha* Dobell?).....Genus *Leidyopsis*

Family 5. **Holomastigotidæ.**

1. Spiral rows of flagella extend from end to end..... 2
   Spiral rows do not extend to posterior end Genus *Microspirotrichonympha*
2. Spiral rows of flagella few in number..... 3
   Spiral rows numerous and close set..... 4
3. Nucleus embedded in dense anterior mass Genus *Holomastigotes*
   Nucleus not in dense anterior mass.Genus *Spirotrichonympha*
4. No spiral ridges; flagella increase in length posteriorly.....Genus *Spirotrichonymphella*
   Many spiral ridges; flagella equal in length Genus *Holomastigotoides*

Family 6. **Teretonymphidæ.**

One genus with characters of the family

Genus *Teretonympha*

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CHAPTER VII.

SPECIAL MORPHOLOGY AND TAXONOMY OF THE SARCODINA.

The term Sarcodina was introduced by Bütschli in honor of Dujardin whose studies on the protoplasm of the Foraminifera led him to believe that the living substance of these forms is simpler than that of other living things and justifying his name for it—sarcode. The peculiarity upon which Dujardin based his conclusion constitutes the essential difference between these types and other groups of the Protozoa. A definite cell membrane is usually absent and the body protoplasm in general is more fluid and more tenuous than in other types. In the absence of confining membranes and with the play of internal forces, the contour of the body is inconstant or constantly changing a phenomenon expressed by the term amœboid movement.

The great majority of Sarcodina are suspended or floating forms (Heliozoa, Radiolaria) and the ground type is homaxonic or spherical, but creeping forms are characteristically flattened, while minor variations of the spherical form lead to the greatest variety of radial ellipsoidal and cylindrical types.

Unlike organisms in the three other great groups of Protozoa the cortex of the Sarcodina rarely shows much structural differentiation. In the majority of cases it is soft and highly vesicular but shows a marked tendency to form an outer or inner lifeless mantle of chitin. Such lifeless mantles or membranes may be tightly fitting or may be in the nature of tests or houses. They may be of pure chitin as in *Cochliopodium*, *Gromia*, etc., or, more frequently, of chitin impregnated with iron oxides, or still more frequently may serve as a substratum on which foreign particles or plates and scales manufactured by the organism, are cemented as in the majority of testate rhizopods. Or between lamellæ of chitin precipitation of calcium carbonate leads to the formation of the limestone shells of the Foraminifera. Skeletons of silica or strontium sulphate of varied patterns and often of exquisite design are characteristic of the Radiolaria, while spicules, rods and plates of silica are widely distributed amongst Heliozoa and Radiolaria.

While many of the Sarcodina are typically uninucleate it may be safely stated that this is exceptional in the group as a whole for the vast majority of Mycetozoa, Foraminifera and Radiolaria are multinucleate. Nuclear dimorphism, however, does not occur and the multinucleate condition is brought about by fusion of cells to

form plasmodia as in the Mycetozoa, or by repeated division of nuclei without accompanying division of the cell as in the Foraminifera and Radiolaria.

Contractile vacuoles are typical of fresh-water forms and their absence is equally typical of salt water and parasitic forms of Sarcodina. When present they are invariably simple and burst directly to the outside without reservoirs, canals or permanent pores, and they furnish the best evidence for the view that contractile vacuoles are primarily regulatory in a physical sense, rather than excretory, in function.

The most characteristic feature of the Sarcodina as a group is the ability of the individual cell to throw out protoplasmic processes called pseudopodia. It was this ability which led Dujardin in 1841 to distinguish these types as *les rhizopodes* from *les flagellées* and *les ciliées*.

Pseudopodia, however, cannot be described by any one definition. The most casual student of the Protozoa will not fail to recognize a difference between the pseudopodia of *Amœba proteus* and those of an *Arcella* or *Difflugia*, while the difference is even more marked between these types and the pseudopodia of any foraminiferon, or between these and any heliozoön. These differences are so pronounced that modern students of the Sarcodina beginning with Lang have distinguished no less than four types of pseudopodia under the names of axopodia, myxopodia, filopodia and lobopodia, and there is some evidence that these four types and in the order given, represent adaptations of a degenerative nature from an ancestral flagellum-like type of motile organ.

Axopodia are homologous with the flagellum of Mastigophora (p. 140). An axial filament extends from the endoplasm to the tip of the pseudopodium. Like the axial filament of a flagellum it is derived from a kinetic element in the endoplasm and as in the hypermastigote flagellates the axial filaments in many forms form the astral rays of an amphiaster at division. In place of the periplastic sheath of the flagellum an axopodium has an investing sheath of ectoplasm in which the protoplasmic granules may be seen streaming back and forth. Many are elastic or mildly vibratile and undoubtedly belong in the category of motile organs since movement of the organism is dependent upon their activity.

Myxopodia are so called because of the tendency to fuse or anastomose when two come in contact. The investing sheath of protoplasm is highly miscible and upon fusion of many pseudopodia a mesh or network, peculiarly characteristic of the Foraminifera, is formed. In this type the axial filament of the axopodia is absent; in its place there is a medullary core of denser substance termed stereoplasmatic axis by Doflein, and interpreted by some as a reminiscence of an earlier axial filament.

Filopodia are homogeneous hyaline pseudopodia possessing in many cases a remarkable elasticity and power of independent movement. It is possible that these pseudopodia do not represent the clear ectoplasm of the Amœba type of pseudopodium, but possibly are homologous with the stereoplasmatic part of a myxopodium, or the highly modified representative of an axial filament.

Lobopodia finally cannot be interpreted properly as motile organs. They are characterized by nothing that can be homologized with structural parts of other types of pseudopodia. They are dependent upon the physical condition of the protoplasm from which they are formed and are present in any type of cell and in any type of animal in which such physical conditions prevail. They are by no means limited to the rhizopods amongst Protozoa but as shown in the last chapter, are characteristic of many types of flagellates as well, and they are formed by one type of cell or another in the majority of higher animals.

It is possible of course that the path of evolution has been exactly the reverse of that outlined above and that progressive evolution has resulted in the gradual differentiation of the more complex types of pseudopodia until with Heliozoa we have a prototype of the Mastigophora. Such an hypothesis makes it more difficult, however, to account for such forms as the Bistadiidæ or the flagellated phases of different types of Sarcodina.

All types of reproduction are represented; simple division, budding division, unequal division and multiple division (p. 208) and the life histories of different types are so variable that a common or generalized account would be inadequate. In general it is legitimate to say that a two-phase, metagenetic, life history is characteristic although certainly not universal. Sexual processes are more widely distributed throughout the sub-phylum than they are in the Mastigophora but here again, these cannot be described as any common type.

Encystment or resting stages are well known in fresh-water forms of Sarcodina but are absent, or have not been described in connection with representatives of the two great groups of marine forms—the Foraminifera and Radiolaria.

Classification of the Sarcodina is fairly well established although minor differences depending upon the individual judgment of relationship in special cases will be found. Division into main groups is made on the basis of pseudopodia types while minor groups are based upon special structural or functional peculiarities. Thus one great group is characterized by the possession of ray-like pseudopodia with axial filaments and is given here the taxonomic value of Class I, the Actinopoda, and these show the nearest approach to the Holomastigidæ amongst the flagellates. A second group—Class II—includes forms with myxopodia, filopodia and lobopodia and is

well termed, in recognition of Dujardin, the Rhizopoda. Possible ancestral types for this group may be found in the Rhizomastigidæ and the Order Chrysomonadida, amongst the Mastigophora.

Class I. **ACTINOPODA**, Calkins.

These are usually homaxonic or spherical forms living for the most part as suspended or floating organisms. Pseudopodia are typically axopodia but lobose pseudopodia may also be formed, mainly as food-taking organs. The protoplasm is highly alveolar, becoming, in the ectoplasm particularly, vesicular or pseudo-alveolar. A highly differentiated cortex is absent as well as the denser cortical protoplasm which characterizes the Amœbidæ. In fresh-water forms (Heliozoa) one or more contractile vacuoles are present in the vesicular ectoplasm. In the Radiolaria, ectoplasm and endoplasm are sharply separated by a continuous chitinous membrane—the central capsule—within which lie one or many nuclei, while the extracapsular protoplasm is differentiated into zones of more or less specialized ectoplasm.

While several types are naked, the great majority of Actinopoda are provided with spicules, plates, spines or skeletons often of elaborate design and exquisite delicacy. For the most part these spicules and skeletons are composed of silica but in one large group of Radiolaria, the Acantharia, they are horn-like and composed of strontium sulphate. According to Dreyer spicules and skeletons depend upon the vesicular configuration of the protoplasm and upon the quantity of mineral matter precipitated between the alveoli (Fig. 11, p. 33).

In Heliozoa a single vesicular nucleus is the rule but there may be from 200 to 300 in *Actinosphærium eichhornii* and several nuclei in *Campionema nutans*. A multiple number is also characteristic of the Radiolaria, or a single nucleus may become enormously enlarged.

Nutrition is invariably holozoic, living organisms being captured through the agency of lobose pseudopodia (Fig. 88, p. 179). Few observations have been made, however, upon digestive processes or final history of the food (see Chapter IV).

Reproduction occurs by division either binary fission or unequal division in the form of budding. Multiple division is frequent in Radiolaria where the endoplasm gives rise to a multiple number of flagellated swarmers which may be of similar or dissimilar size (isospores and anisospores). In some cases both kinds are formed within the same central capsule. Whether these are gametes is a matter, which, while probable, has not been satisfactorily proved.

The Actinopoda are divided into two fairly well-defined sub-classes —the Heliozoa of Haeckel, and the Radiolaria of Joh. Müller.

Sub-class I. **HELIOZOA**, Haeckel.

Heliozoa are typically fresh-water forms although several species of marine forms are known. They are homaxial and floating in habitat for the most part but stalked and attached forms are occasionally met with (*Wagnerella borealis*, *Clathrulina elegans*, etc.). They are either naked (Aphrothoraca) or covered by a gelatinous mantle without spicules (Chlamydophora), or with spicules (Chalarothoraca) or provided with a definite latticed shell (Desmothoraca).

Pseudopodia are typically radial with central axial filaments which penetrate the endoplasm. Here they end, or rather begin, either in a nucleus (*Actinophrys*, *Camptonema nutans*, etc.), or in a central kinetic granule called the Centralkorn by Grenacher (1869) (*Acanthocystis*, *Sphærastrum*, *Wagnerella*, etc.). In such cases the nucleus is excentric. In *Camptonema nutans* a single axial filament arises from each of the many nuclei and there are as many pseudopodia as there are nuclei (Fig. 143). In *Wagnerella borealis* the nucleus is in the basal plate, while the central granule, with radiating axial filaments, is in an enlargement at the other end of the stalk.

The body protoplasm is alveolar and characterized by two zones which in some cases are clearly differentiated as ectoplasm and endoplasm (*e. g.*, *Actinosphærium*) but in most genera they are rather indefinite. The ectoplasm is made up of relatively large pseudo-alveoli in *Actinophrys* and *Actinosphærium* and is very different from the dense ectoplasm of *Amœba*. The endoplasm is more finely granular and contains one or more nuclei (up to two hundred or more in *Actinosphærium*). Symbiotic forms are not infrequent in the endoplasm and are regarded as aflagellate forms of Phytomastigoda.

Contractile vacuoles are present in fresh-water species but are generally absent in salt-water forms. They are developed in the cortex and resemble slightly enlarged ectoplasmic vesicles bursting to the outside.

Nutrition is holozoic, minute lobose pseudopodia being protruded which capture and draw in minute organisms as food. In *Camptonema*, however, the axopodia are able to bend and several of them may be directed toward the capture of living prey (Fig. 143).

Reproduction is ordinarily by binary fission or by budding, while incomplete division frequently leads to colony formation as in *Raphidiophrys*. Sexual processes have been described for only a few forms (*Actinophrys*, *Actinosphærium*) (see Chapter XI) while flagellated swarm spores which may turn out to be gametes, are known for *Acanthocystis*, *Clathrulina* and *Wagnerella*.

If doubtful forms resembling Heliozoa, but without axial filaments (*e. g.*, *Nuclearia*, *Vampyrella*, etc.) are transferred to the Sarcodina

with which they have most affinities, then the classification of the Heliozoa is simple. The division into orders following Hertwig and Lesser (1874) is based upon the absence or the nature of the skeleton elements.

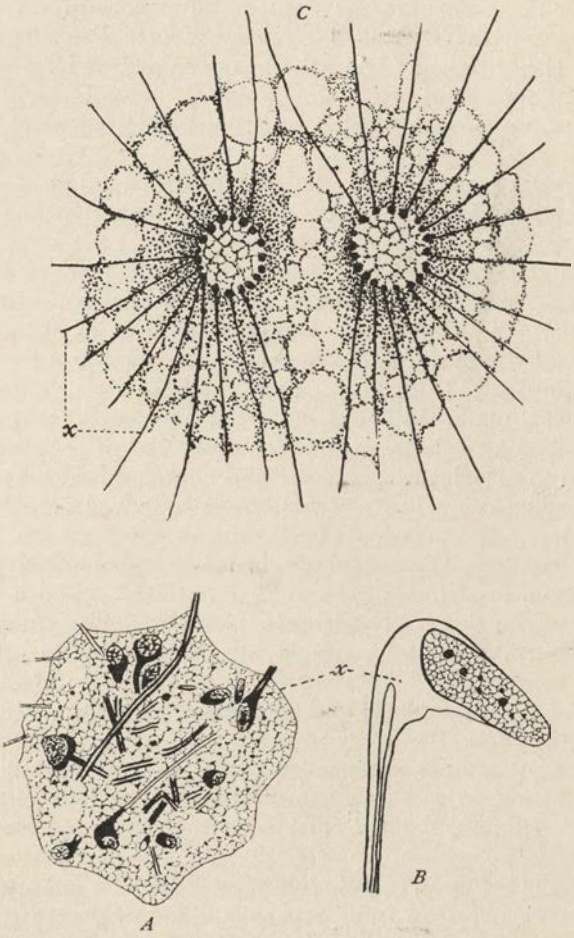

FIG. 143.—*Camptonema nutans* with nuclei partly embedded in the substance of the axial filaments (*A*, *B*,). *C*, Section of *Actinophrys sol* with axial filaments arising from intranuclear granules in recently divided nuclei. (After Schaudinn.)

Order 1. **Aphrothoraca**, Hertwig and Lesser.—Body naked and without gelatinous mantle or spicules. Typical genera: *Actinophrys, Actinosphærium, Myxastrum, Actinolophus, Camptonema.*

Order 2. **Chlamydophora**, Hertwig and Lesser.—Body with a soft gelatinous or felted fibrous covering. Typical genera: *Heterophrys, Sphærastrum, Wagnerella.*

Order 3. **Chalarothoraca**, Hertwig and Lesser.—Body with a gelatinous mantle containing spicules of silica or with a close-fitting skeleton of spicules, spines or plates. Typical genera: *Pomphol yxophrys, Raphidiophrys, Pinacocystis, Lithicolla, Acanthocystis, Diplocystis* (Fig. 144).

Order 4. **Desmothoraca**, Hertwig and Lesser—Body with skeleton shell of one piece perforated by numerous openings. Genus: *Clathrulina.*

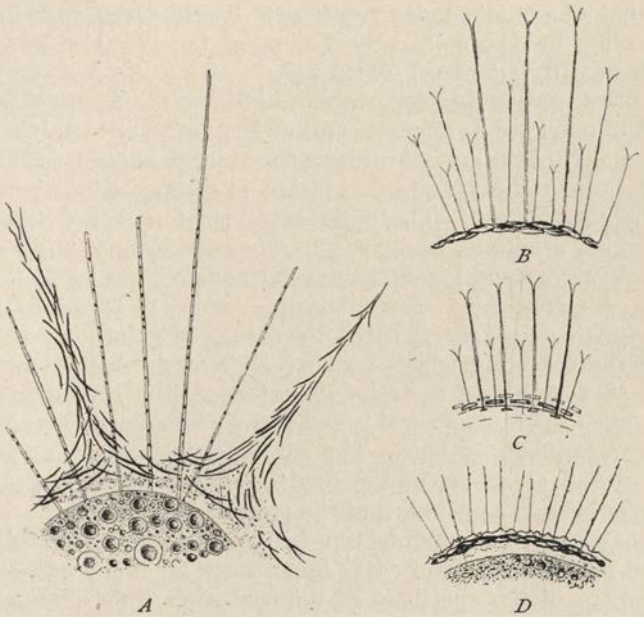

FIG. 144.—Types of spicules in Heliozoa, *A*, *Raphidiophrys pallida* with curved silicious spicules; *B*, *Pinaciophora rubiconda* with tangential plates and forked spines; *C*, *Acanthocystis turfacea*, with separated plates and forked spines; *D*, *Pinaciophora fluviatilis*. (From Calkins after Penard.)

SUB-CLASS II. **RADIOLARIA**, HAECKEL.

Broadly stated the Radiolaria are pelagic organisms of the same general type as the Heliozoa but offer many variations from the homaxonic symmetry of the latter. They are exclusively salt-water forms, surface dwelling for the most part, but may be found at great depths of the sea. Pseudo-alveoli are greatly elaborated and form foam-like spheres with radiating axopodia or with soft protoplasmic pseudopodia-like myxopodia, while complex skeletal elements of silica or strontium sulphate afford the greatest variety of structures and designs.

A typical radiolarian may be conceived by imagining a resistant

membrane of organic substance, presumably chitin or pseudochitin, between the zones of ectoplasm and endoplasm of a heliozoön like *Actinosphærium*. Such a membrane is present in Radiolaria and is called the "central capsule." It separates the intracapsular protoplasm (endoplasm) from the extracapsular protoplasm (ectoplasm). Minute openings, the pylea, through which communication between the two main zones of protoplasm is possible, are uniformly distributed, or arranged in lines and patterns, or limited in number at definite polar positions. These serve as a basis of classification for the main subdivisions of the group according to the scheme early adopted by Hertwig.

The intracapsular protoplasm contains nuclei, fat particles and plastids of one kind or another, and as Verworn showed, it can live independently of the ectoplasm for a time but ultimately regenerates it. The outer or extracapsular plasm is composed of four parts according to Haeckel. The outermost part is a zone of pseudopodia which originate, however, in the more deeply lying fourth zone and then extend through the gelatinous ectoplasm to the periphery. A second zone—sarcodictyum— is in the form of a meshwork which extends through the third zone of gelatinous material termed the calymma which forms the greater bulk of the ectoplasm. A fourth and most important zone, the sarcomatrix lies close against the central capsule and is the go-between for the intra- and extracapsular portions. The sarcomatrix is also the seat of digestion and assimilation, the food coming to it by way of the pseudopodia and the network of the sarcodictyum.

As the means of communication between the central protoplasm and the sarcomatrix is of vital importance to the organism, the arrangement of the apertures in the central capsule offers a good character for the classification of the Radiolaria. Hertwig (1879) who first used this feature, divided the group into four legions as follows: (1) Peripylea in which the membrane of the capsule is perforated by pores arranged regularly around the entire surface; (2) Actipylea, in which the pores are arranged in groups or lines over the surface; (3) Monopylea, in which there is only one such group of pores. In these forms the perforated disc is connected with the center of the central capsule by a conical mass of endoplasm, the podoconus, rich in food particles and granules (Fig. 151 p. 343); (4) Cannopylea, in which the membrane around the pores is drawn out into funnel-like projections termed astropyles of which one is the primary, the other two secondary. In these forms furthermore, the central capsule is double. Haeckel found that certain types of skeleton are characteristic of the different types of membrane perforation and gave corresponding names to the four legions of Hertwig, *viz.*: (1) *Spumellaria*, or practically naked forms; (2) *Acantharia*, with spicules and bars supposed to be of horn or acan-

thin, but later shown by Bütschli to be composed of strontium sulphate; (3) *Nassellaria*, with skeletons and spicules of silica; and (4) *Phæodaria* from the presence of a pigmented mass or phæodium, around the opening of the primary astropyle (see Key, p. 343 for classification).

Class II. **RHIZOPODA**, von Siebold.

With the Rhizopoda we find many more types of structure than are found in the Actinopoda. Myxopodia, filopodia and lobopodia are characteristic but are rarely combined in the same individual. The protoplasm is generally alveolar and may or may not be differentiated into distinct ectoplasm and endoplasm but in general shows less differentiation than in ciliates or flagellates or even in Actinopoda. Protoplasmic inclusions, of the nature of metaplastids, are highly varied while definite plastids are rare. A single chloroplastid in the form of a blue-green chromatophore is present in the testate rhizopod *Paulinella* but these are not known elsewhere in the group. Metaplastids such as "chromatoid bodies" are characteristic of the parasitic amœbæ (Endamœbidæ), while fat and glycogen-like bodies are widely distributed. These are particularly abundant in the fresh-water species *Pelomyxa palustris*, Greeff, the highly refringent bodies "Glanzkörper" found here in abundance are interpreted by Stolç and Bott as glycogen-like in composition, by Veley (1905) as albuminous, and by Goldschmidt (1904) as the plastin remains of nuclei which have broken down with the formation of chromidia. The function of these inclusions and of the accompanying bacteria-like organisms (*Cladothrix pelomyxæ*, Veley) is still a matter of hypothesis. Chromidia, or cytoplasmic chromatin granules, are characteristic and may be permanent constituents of the cytoplasm or periodic (see p. 48).

Living membranes equivalent to the cortical membranes of flagellates, ciliates and gregarines are rarely found here. Transitions toward the chitinous and pseudochitinous tests are present in some forms (*e. g.*, *Cochliopodium bilimbosum*) while the great majority of Rhizopoda have tests of pseudochitin on which mineral substances of quartz, silica, or other types, are cemented. In Foraminifera, calcium carbonate is precipitated between two such membranes of chitin, resulting in the highly complex and multiform shells of lime stone.

Contractile vacuoles are present in fresh-water forms but are generally absent in marine types. They never have the complex canal system such as found in some flagellates and ciliates and are rarely fixed in position. Gas vacuoles are present in some of the testate fresh-water forms (*Arcella*).

The majority of Rhizopoda are multinucleate both in fresh water

and marine species the multiple number due mainly, to repeated nuclear division aided, in Mycetozoa, by plasmodium formation through fusion. The structure of nuclei is too varied for a general description but the vesicular, endosome type predominates (see p. 57).

Nutrition is holozoic and some progress has been made in working out processes of digestion, digestive ferments, etc.(see Chapter IV). Living organisms are captured by pseudopodia or entrapped in the protoplasmic network where they are digested. Cyclosis is invariable and the various protoplasmic granules, digested food substances, etc., are thoroughly mixed.

Reproduction occurs in a variety of ways by division which may be either equal or binary division, budding division, unequal division or budding, and multiple division or sporulation. So-called budding division is the most characteristic and is a form of division apparently limited to the Rhizopoda (see p. 217).

Sexual processes are well developed, microgametes being formed in the majority of cases, which will be reviewed in connection with the several classes.

The classification adopted is an extension of that used by Minchin and includes as primitive forms those questionable Heliozoa-like types which many authors (*e. g.*, Doflein) include with the Heliozoa.

Class Rhizopoda.

Sub-class 1. Proteomyxa.
Naked forms with reticulose or filose pseudopodia.

Sub-class 2. Mycetozoa.
Terrestrial or semi-terrestrial forms characterized by pseudoplasmodium or true plasmodium formation.

Sub-class 3. Foraminifera.
With typical myxopodia and calcareous shells often of complex design.

Sub-class 4. Amœbæa.
Forms naked or with simple one-chambered shells; with lobopodia or filopodia.

Sub-class I. **PROTEOMYXA**, Lankester.

There are but few common characteristics in this group of primitive forms, the most widely spread feature apparently is the usual occurrence of ray-like pseudopodia which recall the appearance of Heliozoa. These have no axial filaments however, and frequently branch or partially anastomose. Flagellated swarm-spore stages are common but the life history is known in few cases. An approach to the Mycetozoa is seen in forms like *Labyrinthula* where the small spindle-shape cells bear long filose pseudopodia which fuse to form a net-like mesh. Most of them are parasites on lower algæ and Protozoa.

Family 1. **Labyrinthulidæ**, Haeckel.—This family is composed of different species of the genus *Labyrinthula* which are intracellular parasites in diatoms, *Vaucheria*, *Spirogyra*, etc. They are always associated in groups or pseudoplasmodia and reproduce by division. Each individual may encyst to form a permanent spore-like resting stage. Flagellated spores are unknown.

Family 2. **Zoösporidæ**, Zopf-Delage.—These forms are also endoparasitic in diatoms, algæ, and various Protozoa, and have filose, Heliozoa-like pseudopodia without axial filaments. They are

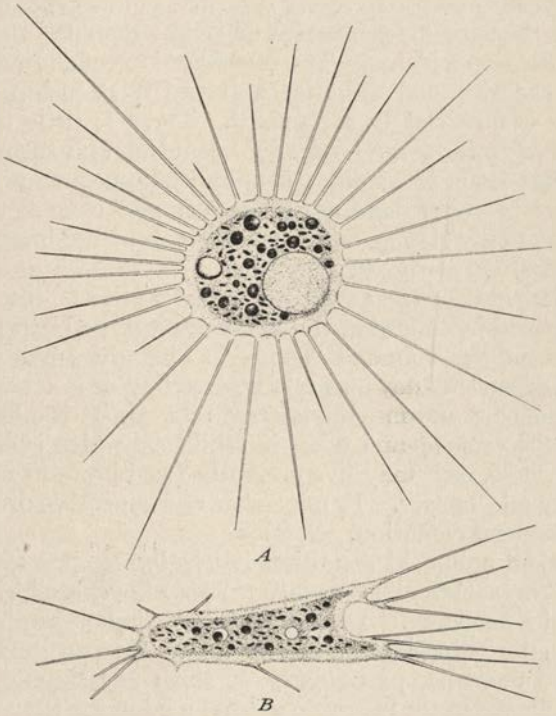

FIG. 145.—*Nuclearia delicatula*, quiescent and moving forms. (From Calkins.)

distinguished by the formation of swarm spores. *Protomonas amyli*, Cienkowsky, apparently lives only on starch grains. Typical genera: *Pseudospora*, Cienkowsky, *Protomonas*, Cienkowsky, and *Protomyxa*, Haeckel.

Family 3. **Vampyrellidæ**, Doflein.—Here also the pseudopodia are very delicate and frequently branch and anastomose and may proceed from all sides of the body or be limited in origin to certain regions. They are frequently parasitic on algæ and Protozoa, some forms having the ability to dissolve the cellulose membranes of

plant cells thus making holes through which they enter the cells. Products of chlorophyll nutrition frequently form reddish-colored masses (karotin) in their protoplasm. Encystment, with cellulose cyst walls is common. Nuclei are multiple as a rule; reproduction by plasmotomy or by division into uninucleate amœbæ; flagellated swarmers unknown. Accepted genera: *Nuclearia*, Cienkowsky; *Arachnula*, Cienkowsky, and *Vampyrella*, Cienkowsky (Fig. 145).

Sub-class II. **MYCETOZOA**, de Bary.

The Mycetozoa were formerly regarded as low types of fungi and under the name of Myxomycetes or "slime moulds" were included amongst the lower plants. The investigations of de Bary, however, revealed the rhizopod affinities and the relationship with other Sarcodina is now clearly recognized. There is little doubt, however, that Mycetozoa are borderline organisms and their semiterrestrial habitat leads to modifications and adaptations not met with elsewhere. Many of them are highly complex both as to organization and as to life history and by no stretch of the imagination can they be regarded as simple organisms.

A general idea of the essential characteristics of the Mycetozoa may be gained by following through a typical life history beginning with a recently germinated "spore." This is a small uninucleate amœboid organism known as a "myxamœba;" it is active, throwing out pseudopodia and moving energetically about the field. It has a contractile vacuole, and takes in solid food which is digested in a gastric vacuole, or it may live upon dissolved proteins from decomposing organic matter. It may also reproduce by division while in this amœboid condition.

The naked amœboid condition is usually temporary; sooner or later the "myxamœba" turns into a "myxoflagellate" by the development of a flagellum. The contractile vacuole is retained and the body, usually ellipsoidal, is highly metabolic and may even give rise to pseudopodia, particularly at the posterior end where the pseudopodia aid in the ingestion of solid food in the form of bacteria, small Protozoa or bits of organic detritus; saprozoic nutrition, however, is also common. Like the "myxamœbæ" the "myxoflagellates" may reproduce by longitudinal division, in which case the centrioles of the mitotic figure become the basal bodies of the flagella. Myxoflagellates are apparently rather sensitive and show a ready tendency to encyst. Such "microcysts" are temporary and the excysted organism again passes through myxamœba and myxoflagellate stages.

According to later investigations of Jahn these myxoflagellates ultimately become gametes; the last division, prior to gamete formation is a chromosome reducing division, and the haploid

gametes fuse to form diploid zygotes. In *Physarum didymoides* the gametes have 8, the zygotes 16 chromosomes.

The zygotes thus formed are very miscible and fusion occurs when two or more come in contact. In this way, and by multiplication of the nuclei by mitosis, and growth, great multinucleated plasmodia arise which may grow to be many inches in diameter and with thousands of nuclei. The former view, based on observations of Cienkowsky, that plasmodia are formed by fusion of myxamœbæ, is now generally abandoned. All observers agree in describing the fascinating spectacle of these sheets of moving protoplasm, a phantasmagoria of living and lifeless granules, nuclei, foreign particles and pigment. The pseudopodia are myxopodia and by their anastomosis great networks of flowing protoplasm form traps for minute organisms utilized as food; some forms, in addition, may be saprozoic in nutrition.

Under conditions which are not entirely known, but some of which are drought and scarcity of food, the entire mass goes into a resting condition. The fluid protoplasm hardens to form a thick walled "sclerotium" which is frequently impregnated with calcium salts. The nuclei collect in groups and these become encysted with cellulose walls. Such resting forms may retain life for some years. Ultimately the hardened walls are liquefied and the plasmodium condition is regained, the process requiring hours or days according to the length of time in the dried state.

With maturity of the plasmodium the gametes, or gametocytes, are formed by processes which are quite remarkable for their intricacy and for the complexity of the specialized structures appearing only at the time of fructification. The whole plasmodium may form one "sporangium," but more often the plasmodium breaks up into several "spore"-forming groups or "sporophores," each from a local heaping of the substance of the plasmodium. Part of such a thickening forms an outer investing wall termed the peridium which is often further hardened by deposition of lime. Another portion becomes differentiated into a thick network or feltwork, termed the capillitium which is continuous with the outer peridium (Fig. 146). This network is made up of tubes and fibers, some of the latter, termed elaters, have a spiral structure and are supposed to function in the distribution of the spores. According to Kränzlin elaters arise from the kinetic components of degenerating nuclei.

The formation of the spores varies in details but the essential part of the process is the fragmentation of the residual mass into uninucleate or multinucleate bits of protoplasm. If multinucleate further fragmentation results in uninucleate bits, each of which encysts independently. According to the later observations of Jahn, the supposed fusion of nuclei leading to the uninucleate condition, and interpreted as autogamic fertilization by Prowazek,

Kränzlin and earlier, by himself, is only a phase in the degeneration of nuclei many of which are disposed of in this way at this period. Fertilization is exogamic, the gametes being the myxamœbæ and myxoflagellates which ultimately emerge from the spores.

Liberation of the spores is accomplished in different ways. In some cases a lid is raised off the sporangium; in others the peridium dissolves in spots leaving a fenestrated capsule; in still others the capsule splits longitudinally. The dry, powdery spores are distributed in various ways, air currents playing a conspicuous part, and they finally germinate in the presence of moisture. Myxamœbæ and myxoflagellates are formed and the cycle is completed.

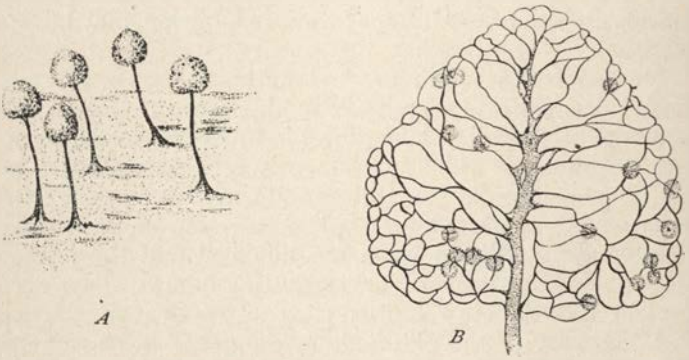

FIG. 146.—Fruiting bodies of *Comatricha nigra*. *A*, five stalked spore capsules; *B*, section of capsule with columella, capillitium, and spores. (After MacBride.)

Genera and species of Mycetozoa are distinguished according to the nature of the plasmodia and by the form and organization of the sporangia.

We follow Doflein in the main grouping of the sub-class Mycetozoa but raise his sub-orders to the value of orders, as follows:

Order I. *Acrasida*, van Tieghem.—In this group the fusion of amœbæ is incomplete but the organisms come together as a gregaloid colony, which is termed here a pseudo-plasmodium. Myxamœbæ are present, but myxoflagellates are not formed.

Order II. *Phytomyxida*, Schroter.—These are parasites in plants and in insects; true plasmodia are formed but peridia and capillitia are absent. Both myxamœbæ and myxoflagellates are characteristic.

Order III. *Euplasmodida*, Lister.—Mycetozoa with myxamœbæ and myxoflagellates and with true plasmodium formation by plastogamic fusion of amœbulæ. The Order includes forms with the full life history as described above.

ORDER I. **ACRASIDA**, VAN TIEGHEM.

(Pseudoplasmodidæ of Zopf-Delage) Sorophora, Lister (in part)

The individual amœboid organisms after a period of creeping by active amœboid movement come together in clusters to form the

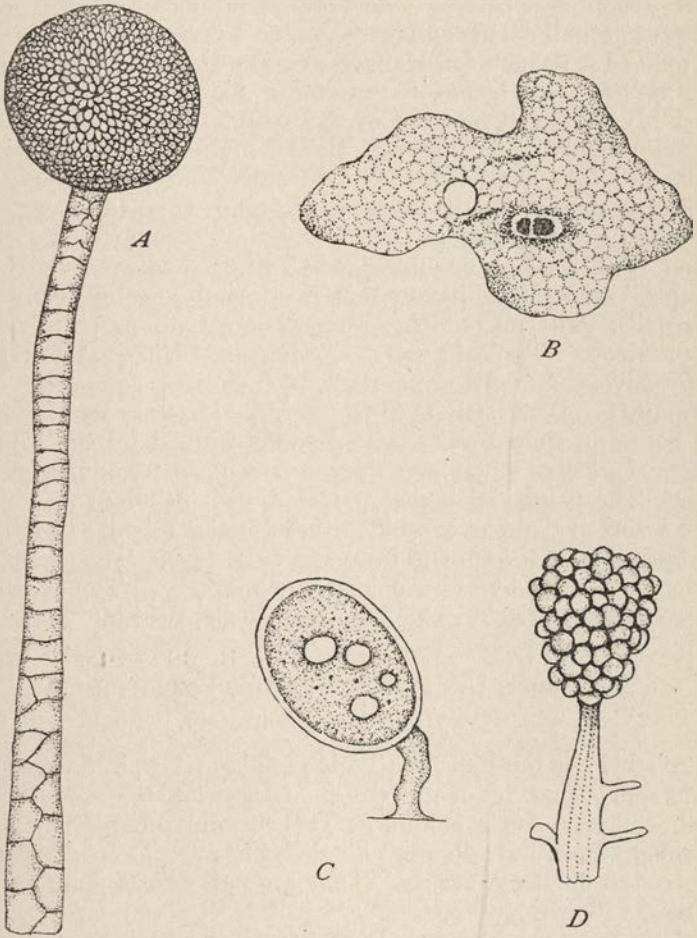

FIG. 147.—*Dictyostelium*, *A*, and *Sappinia*, *B*, *C*, *D*. (After Doflein.)

pseudoplasmodia, the amœbæ retaining their individuality. Individuals creep up over their fellows and form groups or sori which in some cases are stalked, the stalks being formed by the dried bodies of sacrificial *amœbæ*. The sori are formed by other amœbæ creeping over the stalk and accumulating in a mass at the top. Here each

encysts and when a suitable medium is assured the small amœbæ again creep out, often, however, after a long period of desiccation. Their characteristic habitat is animal dung.

While many competent authorities regard these organisms as remotely related, if at all, to the more complex Mycetozoa, we believe that their affinities are more probably here than with any other group of Protozoa. The three families recognized show different gradations in complexity.

Family 1. **Sappiniidæ**, Dangeard.—The single genus and species —*Sappina pedata*, Dangeard— forming this Family differs from all other Mycetozoa in that not even a pseudoplasmodium is formed, a single amœba going through all the motions of a plasmodium. Stalk and cyst are formed by one individual but the cysts are frequently massed in sporangium-like groups (Fig. 147).

Family 2. **Guttulinidæ**, Cienkowsky.—These are small forms which bear stalked or unstalked fruiting bodies covered with "spores." The latter have either thin membranes or heavy cellulose walls. The myxamœbæ foregather in clumps on which the sori originate. Typical genera: *Guttulina*, Cienkowsky, *Guttulinopsis*, Olive.

Family 3. **Dictyostelidæ**, Rostafinsky.—Here the fruiting bodies are borne on simple or branched stalks formed by the hardened bodies of amœbæ which have migrated from the pseudoplasmodium mass. The polygonal bodies, covered with cellulose membranes, form a sort of tissue over which other amœbæ migrate to form sori at the top or at the ends of branches (Fig. 147). The myxamœbæ are characterized by thin, pointed pseudopodia. Typical genera: *Dictyostelium*, Brefeldt, and *Polyspondylium*, Brefeldt.

Order II. **PHYTOMYXIDA**, Schroter.
(Phytomyxinæ, Schroter).

Probably as a result of parasitism peridia and capillitia are absent in the representatives of this group. Otherwise they agree with the more complex Euplasmodida. They form true plasmodia and myxoflagellates, but there are no closed sporangia, recalling in this respect the simpler Acrasida. They are parasitic in plant cells and in insects (beetles).

Plasmodiophora brassicæ, Woronin, is the best known of this group largely because of its economic importance. It attacks the roots of cabbages and other Cruciferæ and produces a characteristic tumor disease known as "Club-root," "Hanberries," "Fingers and Toes," "Kohlhernie," etc.

Minute flagellulæ are formed from the cysts in an infected garden and these, in some way, penetrate the root cells of the plant and become myxamœbæ. The nuclei multiply and they grow in the cells

of the plant, different individuals fusing to form plasmodial masses which fill the cell. With exhaustion of the cell contents the process of reproduction begins and results in the formation of great masses of uninucleate "spores."

Other genera parasitic on plants are *Tetramyxa*, Goebel (forming galls on *Ruppia rostellata*) and *Sorosphæra*, Schroter (causing tumors in various species of *Veronica*).

The genera *Sporomyxa*, Léger, and *Mycetosporidium*, Léger and Hesse, are parasites of beetles (*Scaurus tristis*, and *Otiorhynchus uscipes*).

Order III. **EUPLASMODIDA**, Lister.
(Mycetozoa s. str. Myxogasteres).

This order includes the great majority of Mycetozoa and forms which in their life histories agree with the description given above (p. 326). Myxamœbæ and myxoflagellates are invariable, so too are true plasmodia and complex sporangia which with the exception of the family Ceratiomyxidæ (Exosporea), are invariably surrounded by a peridium.

The "spores" are usually globular, rarely elliptical and are often compressed by pressure into polygonal forms. In the majority of cases they are violet in color but colorless, white, yellow, brown and red sporangia are known. In most cases the "spores" are uninucleate but forms with two, and with four nuclei are known.

In some cases the simultaneously formed sporangia unite to form a common fruiting body in which the individual sporangia may still be distinguished in some types. In other types, however, this independence is lost and one common fruiting body results, with one continuous capillitium. Such fruiting bodies are called æthalia. See Key for further classification.

Sub-class III. **FORAMINIFERA**, d'Orbigny.
(Reticulosa, Thalamophora).

This group of the rhizopods includes a large number of bottom dwelling, marine Sarcodina with anastomosing pseudopodia (myxopodia). A few forms live in fresh water (*Allogromia* species), and some forms are pelagic in the sea (*Globigerina*, etc.). The great majority are provided with shells or tests composed for the most part of calcium carbonate. In some, however, the shell is purely organic, consisting of substance of gelatinous or pseudochitinous character (*Allogromia*); or foreign particles of sand, diatom shells and detritus of one kind or another, may be cemented to the pseudochitinous test by gelatinous or chitinous cement. Such tests are usually described as arenaceous, in contrast with the clear lime shells or porcellanous types. The walls of the shells are either thick and

homogeneous or are perforated by minute pores (foramina) through which single pseudopodia are protruded. The cavity of the shells may be a single chamber, septa if present being incomplete (Monothalamous). Or a multitude of chambers may be present separated by partitions or septa (polythalamous). The latter may be complicated by secondary deposits of lime through which labyrinthine canals and passages give occasion for intricate designs (Fig. 148). The surfaces of the shells are usually smooth but in some forms particularly the floating types of *Globigerina*, spines, ridges, rays, etc., probably assist in floating.

The living substance is usually so fluid that it is rarely quiet and protoplasmic streaming is so characteristic that the Foraminifera

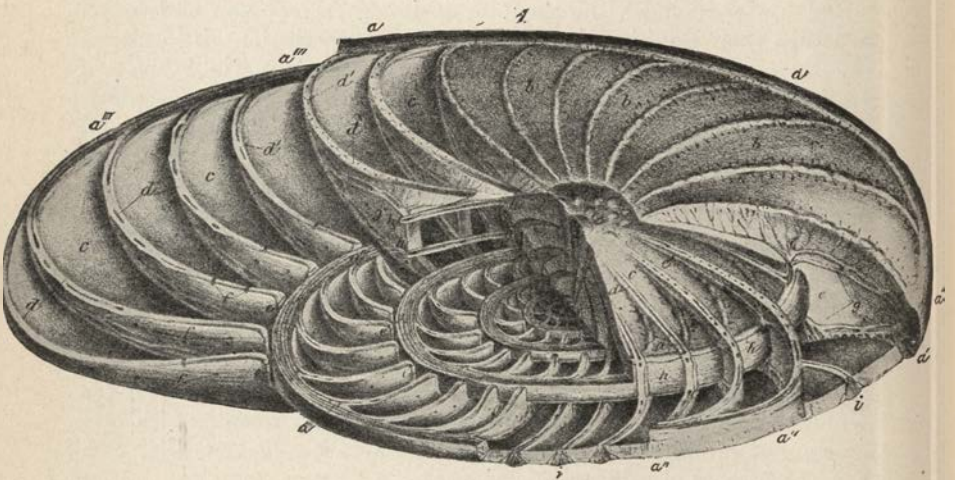

FIG. 148.—Polythalamous shell of *Operculina* (schematic). The shell is represented as cut in different planes to show the distribution of the canals and the arrangement of septa and chambers. (After Carpenter.)

have been favorite materials for the study of protoplasm. It is not divided into zones, and the marine forms have no vacuoles. There are numerous foreign bodies as a rule and aggregates of the residue associated with food substances, form masses of fecal material termed "stercome." In many forms living commensals are also present in the form of small yellowish Cryptomonas-like forms which are liberated with sporulation of the host organism (*Chrysidella*).

The living protoplasm fills more or less completely all chambers of the organism. In polythalamous forms protoplasmic strands passing through pores in the septa maintain all parts of the soft body as a unit mass. In monothalamous and from the last-formed chamber of polythalamous forms, a large mass of protoplasm gives

rise to the pseudopodial network which acts as a trap for the capture of diatoms, crustacea, rotifers and other smaller objects used as food. In the perforate types pseudopodia are also protruded through the finer pores (foramina) of the shell.

One large vesicular nucleus is characteristic of both single and many-chambered types. In the latter the nucleus may be confined to the first formed, or inner, chambers, although it may wander throughout the entire organism. In many cases it is replaced by

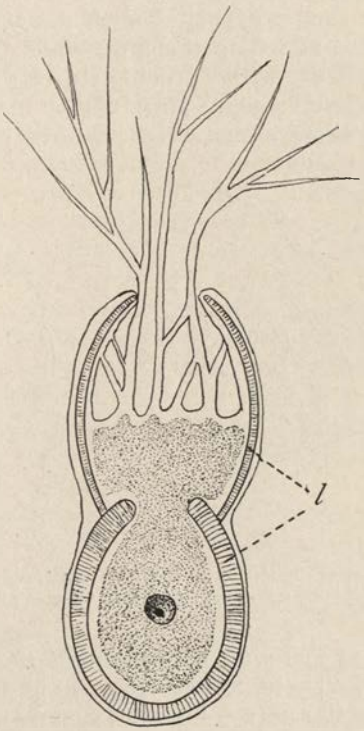

FIG. 149.—Diagram to show the mode of origin of the Nodosarine type of Foraminifera shell.

several nuclei, and there is a general tendency throughout the group to form chromidia by multiple division, or fragmentation of the primary nuclei.

Reproduction may or may not be accompanied by fertilization phenomena and throughout the group there is a more or less regular alternation of sexual and asexual processes, accompanied in many cases, by morphological evidence of sexual or asexual generation. In its simplest case, asexual reproduction consists of so-called budding division. In *Allogromia* for example, the protoplasm streams

out of the shell mouth and forms a ball of protoplasm of about the same size and shape as the parent organism; on the extruded bud a daughter shell is secreted and after division of the nucleus and migration of one of the daughter nuclei, the bud becomes detached and begins an independent existence (Fig. 149). In the polythalamous forms, an initial shell of one chamber contains an organism which grows and buds in a similar manner, but the bud does not become detached. According to the type of budding shell types known as Nodosarine, Frondicularian and Rotalian, are formed (Fig. 150). A new shell is deposited about the naked bud and thus a second chamber is added to the first, while the protoplasm by division of the nucleus, without complete cell division, becomes binucleated or multinucleated. In a similar manner other chambers are added to those already formed until complicated aggre-

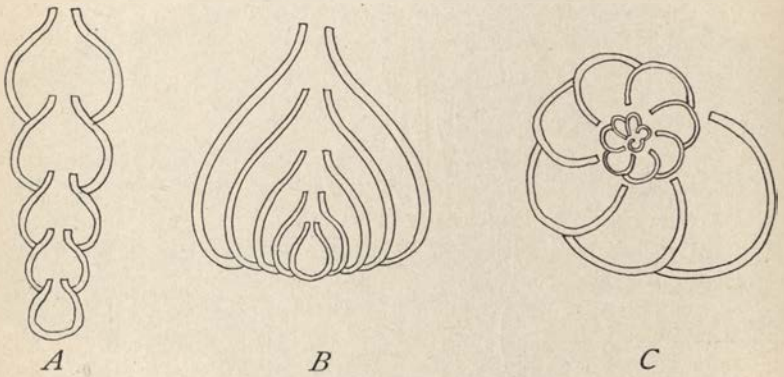

FIG. 150.—Types of polythalamous Foraminifera shells. *A*, Nodosarine type; *B*, frondicularian type; *C*, spiral type. (From Calkins after Carpenter.)

gates measuring 3 or more inches in diameter in some cases, result (*Nummulites*, etc.). These, however, are to be regarded as single individuals of syncytial nature illustrating growth and differentiation rather than reproduction. With the formation of a brood of reproductive bodies each of which produces a similar multinucleated individual we can speak of asexual reproduction in a strict sense. Thus in *Polystomella crispa* (Fig. 119, p. 239), after multiplication of the nuclei, the latter give rise by fragmentation to a large number of minute nuclei having the significance of chromidia. The plasm forms islands about each of these minute nuclei, or groups of them, and is then broken up into as many minute cells as there are islands. These small cells, in the form of amœbulæ or amœbospores leave the parent shell by way of the foramina or by the mouth opening of the last chamber and after a short period of amœboid movement settle down and secrete the characteristic shell chamber. This

initial shell is measurably larger than the initial chamber of the organism which formed the amœbulæ and is called a macrospheric chamber as opposed to the microspheric chamber of the first generation. A new multi-chambered shell is then formed according to the type of structure of the species. When fully grown the protoplasm of this macrospheric generation breaks up into a swarm of small biflagellate flagellispores which leave the parent shell and swim about by means of their flagella. These flagellates are gametes which ultimately unite two by two to form zygotes. The flagella are absorbed and the young zygotes secrete the shell material of the first chamber about which other chambers are formed with growth and budding division until the mature individual again results. Thus there is a typical alternation of generations in the life history of a foraminiferon; the microspheric individual starting from a zygote, with its production of amœbulæ is an asexual generation while the macrospheric individual starting from an asexual spore, is the sexual generation giving rise to gametes. In *Polystomella* the relative abundance of macrospheric and microspheric shells is 38 to 40 of the former to 1 of the latter (Rhumbler, 1923).

In classification, the form of the shell is usually given the first importance while the substance of which the shell is composed is secondary. There are so many types and variations of type in the group that generalizations, useful for taxonomic purposes, are difficult. Certain plans of structure representing modes from which variations appear, are evident however. Rhumbler recognizes five of these main types as follows:

1. The nodosaroid type: With chambers arranged one behind the other in a straight or slight curved line (Fig. 150).

2. The spiral type: With chambers arranged in such a way that an imaginary line passed through the mouth openings of the successive chambers would be a spiral line.

3. The cyclical type: With later formed chambers arranged as circles about the initial chambers, the circular chambers being further subdivided into small secondary chambers.

4. The azerval type: The earlier chambers are arranged in a spiral but the later chambers are heaped in an irregular mass.

5. The textularid type: The chambers are arranged in one or two linear rows. For classification see Key p. 353.

Sub-class IV. **AMŒBÆA.**

When rhizopods are mentioned the mental picture in most cases is *Amœba* or some of its close relations amongst the Amœbæa. It is not the largest group of rhizopods but some of the forms included here are amongst the most common types of Protozoa, while their apparent simplicity and enigmatic movement have given them the

popular position of the lowest forms of animal life and the phrase "from Amœba to man" is familiar to everyone. They are present in all stagnant fresh and brackish water; in damp moss or leaves; abundant in the superficial soil, and also abundant as commensals or parasites in all kinds of animals.

In all of the naked forms there is a well-marked differentiation of the protoplasm into endoplasm and ectoplasm. The latter is more dense, the former more fluid and with typical cyclosis. In the shelled types there is frequently a characteristic zonal differentiation.

Pseudopodia are never myxopodia or axopodia. Naked forms have blunt finger-form processes or lobopodia formed by an outflow of ectoplasm and endoplasm. Shelled forms in the majority of types, have pseudopodia composed apparently of ectoplasm only. These have considerable power of movement apart from the usual amœboid type of flowing substance, and may sway or move independently with vigor. In the naked forms pseudopodia may be thrown out from any part of the cell, but in shelled types they are limited to the region adjacent to the orifice of the shell. In some cases, as in the genus *Cochliopodium*, there is a firm ectoplasm which has many of the features of a chitinous membrane. Pseudopodia pass through it by means of permanent apertures (Fig. 8, p. 30). and when the cell divides the membrane also divides. There are very few of such forms, however, the great majority of shelled forms having a definite chitinous membrane on which foreign particles are attached. In Arcellidæ the membrane is clear chitin and in the Euglyphidæ the outer elements of the shell are secreted before division and passed out to the daughter individual after the chitin membrane is laid down. The variety of shells is due to the different types of sand crystals, diatoms, detritus of various kinds and even living plants cells.

The nucleus is vesicular and usually single although many types of both naked and shelled forms are binucleated or multinucleated. The entire group is further characterized by the distribution in the cytoplasm, of granules of chromatin which originate from the nucleus.

With the exception of the parasitic forms, and some of these are also included, the Amœbæa are holozoic in nutrition and proteolytic and amylolytic ferments have been isolated in some cases (see Chapter IV).

Notwithstanding the abundance and the wide distribution of these forms of rhizopods there is very little agreement on the part of different observers in regard to the life history. Few Protozoa have been more frequently seen and studied than *Amœba proteus* and yet nothing is known accurately about the life cycle. Binary division is characteristic of all the naked forms both free-living and parasitic, and encystment stages are known in all forms. So-called

budding division is typical of the testate forms and differs materially from binary fission (see p. 217). Acceptable accounts of sexual processes are limited to the Testacea in which there is a general resemblance to the type of gamete formation characteristic of the Foraminifera (see Chapter V).

Parasitic forms of the Amœbidæ are widely distributed throughout the animal kingdom. They are usually present in the digestive tract but may be ectoparasites as well. The great majority are of the nature of commensals and are harmless, some, however, are pathogenic as *Amœba mucicola*, Chatton, a harmful ectoparasite on the gills of Labridæ, or *Endamœba dysenteriæ, Craigia hominis,* and *Councilmania lafleuri*, Kofoid, causes of dysentery in man.

The organisms included in the Amœbæa fall naturally in one of two groups which have been generally recognized as Amœbida (Gymnamœbida) and Testacea. Following the principle adopted in classifying the Mastigophora where amœboid forms of animal flagellates are retained as Mastigophora only when the flagellum or flagella are permanent structures of the organism, we include as rhizopods those forms with pseudopodia and temporary flagella; flagella and pseudopodia being more or less interchangeable. These are included here in the family Bistadiidæ of Doflein.

Order 1. **Amœbida (Gymnamœbida),** Ehrenberg.

Naked forms of amœbæa, either free-living or parasitic; with one or more nuclei; with contractile vacuole (except in some of the parasitic forms); reproduction by binary fission, multiple division occasional. Encystment widespread.

We recognize four families in this order, *viz.:* Bistadiidæ, Amœbidæ, Endamœbidæ and Paramœbidæ. Separation of the parasitic forms of amœbæ from free-living forms is hardly justifiable in a natural classification but is tolerated on grounds of expediency.

Family 1. **Bistadiidæ,** Doflein.—Organisms characterized by two interchangeable phases—amœboid and flagellated. In the former phase the body is amœboid with lobose pseudopodia. A single nucleus with endobasal body is present; the basal body of the flagellum is formed by division of the endobasal body (Wilson) and the flagellum grows out from the basal body. Transformation from the amœboid to the flagellated condition involves loss of amœboid movement and change in form to a monaxonic ellipsoidal form. Absorption of the flagellum accompanies transformation again to the amœboid condition. These changes are evidently induced by environmental conditions and, in cultural forms, may be brought about at will. Genera with one, two, and three flagella in the flagellate phase are known. Reproduction by division limited to

the amœboid phase, sexual processes unknown. The amœboid phase is represented by small creeping amœbæ which have been generally included as *Amœba limax*, and known as "limax" forms. These were separated from the genus Amœba by Chatton and Lalung-Bonnaire (1912) under the name *Vahlkampfia*. The forms with a single flagellum in the flagellated stage are retained under the generic name *Vahlkampfia* although it is by no means assured that all "limax" amœbæ are thus dimorphic. Forms with two flagella are grouped in the genus *Nägleria* and forms with three flagella in the genus *Trimastigamœba*, Whitmore. Parasitic forms, regarded by Craig (1906) as a cause of human dysentery and with a flagellated phase with one flagellum, are included in the genus *Craigia*. Genera recognized: *Vahlkampfia*, Ch. and L. B., type *V. bistadialis* Puschkarew (Fig. 42, p. 86); *Nägleria*, Alex.; type *N. gruberi*, and *N. punctata*, Dangeard (Fig. 12, p. 34); *Trimastigamœba*, Whitmore; type *T. philippinensis*; *Craigia*, Calks.; type *C. hominis*.

Family 2. **Amœbidæ** (authors generally: em. Doflein, em. Calkins).—The usual types of free living amœbæ are grouped in this family. Flagella, so far as known, are absent at all stages. Nuclei single, double, or multiple; contractile vacuole usually single, present generally in fresh-water forms. Reproduction by simple division in vegetative forms, by multiple division during quiescent phases. The great majority of forms are aquatic and developmental phases of other types (*e. g.*, mycetozoa) may be easily mistaken for amœbæ. Others are semi-terrestrial living in damp earth, moss, etc., where they play a part in keeping down bacteria of the soil (see Goodey). Sexual processes in no case have been substantiated, but a peculiar process of syngamy has been described by Nägler, Erdmann and others in the case of *Amœba diploidea* (see p. 549). Genera: *Amœba*, Ehrenberg; *Pelomyxa*, Greeff.

Family 3. **Endamœbidæ.**—These are parasitic amœbæ widely distributed throughout the animal kingdom and with characteristic vegetative phases during which the organisms live as harmless commensals, or more rarely, as pathogenic parasites in the host, and with permanent cyst stages by which infection is carried by means of contaminative infection. The genus generally recognized is represented by a vast number of species with ill-defined diagnostic characters (genus: *Endamœba*) while other genera (*e. g.*, *Endolimax? Councilmania*, etc.) are forms about which the taxonomic position is still in dispute. Nutrition is either holozoic, saprozoic or heterozoic.

Family 4. **Paramœbidæ.**—Forms with single nucleus and peculiar cytoplasmic structure (Nebenkern) variously interpreted as a kinetic element, intracellular parasite, etc. Both free-living and parasitic species. Genus: *Paramœba*.

Order 2. **Testacea.**

These forms are generally described as amœbæ with shells; by some they are grouped as a subdivision of the Foraminifera (Doflein). The protoplasmic and test structure, as well as the pseudopodia are so different from Foraminifera that little is gained by this procedure, while the association with naked forms has a long historical backing. They are almost exclusively fresh-water forms, although some species are represented in brackish water as well. Many species are semiterrestrial and abound in moss and similar damp places. The protoplasmic body differs from that of the Amœbidæ in having the ectoplasm concentrated at the region of the shell opening, while many forms show a distinct zonal differentiation of the protoplasm. Contractile vacuoles are always present.

Nuclei are either single, double or multiple and are usually accompanied by a zone of chromidia in the form of a dense reticulum from which, according to the observations of numerous observers, the nuclei of gametes are formed (Schaudinn, Zuelzer, Elpatiewsky, *et al.*). It is rather the fashion to doubt this interpretation on the ground that such nuclei are probably parasites, but we shall adhere to it until the critics have a more probable explanation of the nature of the chromidia (p. 48).

Pseudopodia are filopodia which in a few instances, have the tendency to branch. They lack the medullary endoplasm of lobopodia and have a considerable power of independent movement.

The tests are simple, one-chambered structures of widely-varied form, frequently ornamented with spines and processes. The basis of all shells is a pseudochitinous membrane which, in some forms is greatly thickened and constitutes the test; in other cases foreign particles are cemented to the outside of the chitinous membrane (*Difflugia*, *Centropyxis*, etc.), and in still other cases silicious plates are precipitated in the endoplasm in the vicinity of the nucleus, and deposited on the chitinous membrane in definite patterns characteristic of different genera (*Euglypha*, *Quadrula*).

Reproduction occurs by longitudinal binary division in forms with a soft chitinous membrane, where membranes divide with the soft body; in other cases it occurs by so-called "budding division" whereby the protoplasm swells out of the shell mouth to form a bud which assumes the size and shape of the parent (p. 217). Multiple division also occurs in some types; many nuclei are formed by division; these become the nuclei of small naked amœbæ which after a short period of free movement and growth secrete the shell characteristic of the species. Fertilization processes have been described for several types (*Centropyxis*, *Arcella*, *Trichosphærium*, *Difflugia*, etc.), the gametes being either amœbulæ or flagellulæ.

A typical alternation of generations comparable with that of the Foraminifera was described by Schaudinn for the peculiar genus *Trichosphærium*. Here asexual processes occur by irregular plasmic divisions (plasmotomy) and by multiple division resulting in a swarm of minute naked amœbæ. These develop into an adult form of different type which may likewise undergo plasmotomy leading to the formation of gamonts and gametes. The latter, upon fertilization, give rise to the initial type of organism. In this cycle, the original asexual generation differs from the later sexual generation by the presence of a peculiar type of test consisting of radially-arranged spicules of magnesium carbonate.

The forms included in this Order fall naturally into two families—Arcellidæ and Euglyphidæ (see key for genera).

Family 1. **Arcellidæ.**—Tests transparent or opaque by reason of covering of foreign bodies picked up by the protoplasm and deposited on the outside where they are cemented to the chitinous membrane.

Structure and materials of the shell afford a basis for further classification of the family. They are either pyriform or shaped like a watch glass; the membrane may be rigid or flexible and the aperture central or asymmetrically placed.

Sub-family *Arcellinæ*.—The tests are watch-glass shape, membranous or chitinous in character. A definite aperture is wanting in *Pseudochlamys*, the test resembling an inverted saucer over the organism. A central aperture is present in the other genera except in *Centropyxis* where it is asymmetrically placed. It occupies most of the ventral surface in *Pyxidicula*, but is relatively small in others and variously modified: (1) *Arcella* with margin of the test inturned at the oral opening; (2) without the inturned collar in *Capsellina;* with a velum or distensible membrane about the aperture as in *Microcorycia;* (3) with aperture slit-like instead of circular, as in *Parmulina*. In one genus: *Diplochlamys* the membrane is double, the outer bearing foreign particles.

Sub-family *Difflugiinæ*.—Here the tests are pyriform, not compressed, and covered with foreign particles of diverse kinds. They may be arcelliform but asymmetrical and with an arcuate aperture as in *Bullinula;* or spheroidal with a narrow slit-like mouth as in *Plagiopyxa;* or even spiral, with a distinct neck as in *Lesquereusia*. The majority of tests are symmetrical, in some forms with a long neck as in *Cucurbitella;* in others an internal diaphragm forms a neck-like constriction as *Pontigulasia*. Large pyriform types with different kinds of foreign bodies, and opaque, are included in the genus *Difflugia*, while smaller forms of similar shape, but somewhat compressed and transparent, are placed in the genus *Cryptodifflugia*. Pseudopodia are generally of the filopodia type but in *Difflugiella*, both lobopodia and filopodia are present, while in *Phryganella*, the

pseudopodia are blunt at the base but provided with long sharp points.

Sub-family *Nebelinæ.*—Here the tests are transparent and dome-shaped except for lateral compression. They are homogeneous and transparent in *Hyalosphenia* but are covered with scales, plates, etc. in other genera. A definite thickening of the shell about the aperture is typical of *Awerintzia.* In *Heleopera* the crown of the test has a few sand grains adherent, while in *Nebela* and *Quadrula* the test has an outer covering of scales which are quadrangular and arranged in oblique rows in *Quadrula,* and are circular, oval or irregular in *Nebela.*

Sub-family *Pseudonebelinæ.*—Here the test is usually flexible either in the region of the aperture only, as in *Amphizonella,* or throughout as in *Zonomyxa* (which is multinucleate) and *Cochliopodium* (uninucleate). In *Leptochlamys* only, is the test rigid, structureless, and transparent, and with a peculiar ball-like single pseudopodium.

Family 2. **Euglyphidæ.**—In members of this family the test is covered by silicious plates or scales and the pseudopodia are of a filose, branching type. The tests may be either symmetrical or asymmetrical. In the former group the aperture is terminal, circular and provided with teeth in *Euglypha* formed from scales; or the edge of the aperture is smooth or slightly serrated in *Sphenoderia.* In asymmetrical forms the mouth is subterminal, and oblique in *Campascus.* The test is retort-shape in *Cyphoderia, Campascus* and *Nadinella.* It is pyriform but much compressed in *Placocista* (without toothed membrane) and *Assulina* (with toothed membrane about the aperture). In *Paulinella* the test is *Euglypha*-like but the cell body possesses a band-form blue-green chromatophore. In *Trichosphærium,* finally, there is no definite test but the body is enclosed in a gelatinous mantle with radial rods in the asexual generation and without these in the sexual generation.

III. KEY TO GENERA OF SARCODINA.

Pseudopodia with axial filaments—axopodia
Class 1. Actinopoda
Pseudopodia without axial filaments. Class 2. Rhizopoda

Class 1. **ACTINOPODA,** Calkins.

Marine forms; with central capsule
Sub-class 2. Radiolaria
Mainly fresh water; no central capsule
Sub-class 1. Heliozoa

Sub-class 1. **HELIOZOA,** Haeckel.

Naked forms; no jelly mantle or skeleton
Order 1. Aphrothoraca

With gelatinous mantle; no silicious spicules
Order 2. Chlamydophora
With isolated or united spicules.....Order 3. Chalarothoraca
Skeleton globular; silicious; many openings
Order 4. Desmothoraca

Order 1. **Aphrothoraca,** Hertwig.

1. Individuals without stalk..... 2
   Individuals with stalk..... 5
2. Ectoplasm and endoplasm distinct..... 4
   Ectoplasm not distinctly differentiated... 3
3. Axial filaments extend to central nucleus
   Genus *Actinophrys*
   Each axial filament ends in a nucleus
   Genus *Camptonema*
4. Axial filaments end with ectoplasm. Genus *Actinosphærium*
   Axial filaments end in centroblepharoplast
   Genus *Gymnosphæra*
5. Stalk contractile.....Genus *Zoöteira*
   Stalk not contractile..... 6
6. Stalk hollow.....Genus *Actinolophus*
   Stalk solid.....Genus *Haeckelina*

Order 2. **Chlamydophora,** Archer.

1. Mantle without foreign bodies..... 2
   Mantle with foreign bodies..... 3
2. Mantle granular; radial surface markings
   Genus *Heterophrys*
   Mantle smooth; radiating fibrils; rough
   Genus *Sphærastrum*
3. Plasm with colored oil globules ....Genus *Eleorhanis*
   No oil; mantle with one layer of sand grains
   Genus *Lithocolla*

Order 3. **Chalarothoraca,** Hert. and Less.

1. Without stalk..... 2
   With stalk..... 3
2. Membrane with minute spherical granules
   Genus *Pompholyxophrys*
   Membrane with circular discoidal plates
   Genus *Pinacocystis*
   Membrane with pointed oval plates. Genus *Pinaciophora*
   Membrane with tangential, loose, needles
   Genus *Raphidiophrys*
   Membrane with radially arranged spines
   Genus *Acanthocystis*
3. Membrane with small plates.....Genus *Cienkowskya*
   Membrane with radial spines.....Genus *Wagnerella*

Order 4. **Desmothoraca,** Hert. and Less.

1. With stalk..... 2
   Without stalk..... 3
2. Capsule spherical; openings large...Genus *Clathrulina*
   Capsule polygonal; openings small..Genus *Hedriocystis*
3. Capsule spherical; pores with collars Genus *Choanocystis*
   Capsule spherical; no collars.....Genus *Elaster*

Sub-class 2. **RADIOLARIA**, Joh. Müller.

The great number of genera of Radiolaria make it impossible to give more than a superficial survey of this group. The four Legions of Hertwig and of Haeckel are regarded as Orders in the following description, and differ according to the arrangement of pores (pylea) in the central capsule (Fig. 151).

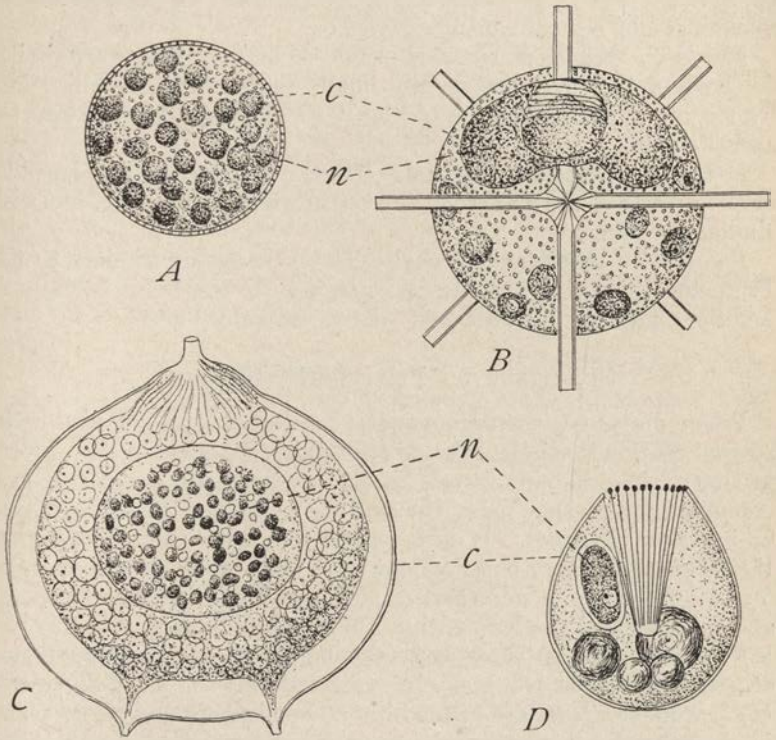

Fig. 151.—Radiolarian central capsules. *A*, *Thalassolampe*, type of peripylea; *B*, *Acanthometron*, type of actipylea, *C*, *Aulographis*, type of tripylea; *D*, *Tripterocalpis*, type of monopylea; *c*, central capsule; *n*, nucleus. (From Calkins after Haeckel.)

Order 1. **PERIPYLEA**, Hert.

The Peripylea (also called Spumellaria) are characterized by the possession of a spherical central capsule with pores distributed uniformly; a skeleton is generally absent or represented by scattered spicules; colony formation is frequent, the colonies often measuring several millimeters (Collozoum) or even centimeters. Nuclei are multiple as a rule, but in some types a single huge nucleus apparently represents the aggregate of nuclei in the multinucleate forms and is regarded as a polyenergid by some writers (Hartmann, *et al.*).

Following the majority of recent writers we divide the Peripylea into three sub-orders—Sphærellaria, Polycyttaria, and Collodaria.

Sub-order 1. **Sphærellaria,** Haeckel.

Small forms with a rather complex latticed skeleton or closely associated single elements, always of silica.

Family 1. **Sphæroidæ**, Haeckel.—The skeleton is latticed and may be multiple and concentric as in Cenosphæra or Staurosphæra. Skeletons and central capsules globular.

Family 2. **Prunoidæ**, Haeckel.—The skeleton elements are similar to those of the preceding family but the organisms are ellipsoidal by reason of the elongation of the vertical axis of central capsule and skeleton.

Family 3. **Discoidæ**, Haeckel.—These are flattened and lens-like or the reverse of the axial relations of the preceding family, but with similar skeletons.

Family 4. **Larcoidæ**, Haeckel.—These forms are similar to the Prunoidæ in having the vertical axis longer than the horizontal, but the organisms are flattened "dorso-ventrally."

Sub-order 2. **Polycyttaria,** Haeckel.

When mature these organisms are colonial, the colonies often several centimeters in length and ellipsoidal, spherical, or band-form in shape. A great number of central capsules are embedded in a common protoplasmic mass, the capsules being increased by division or budding (Brandt). It is in connection with these forms that the most satisfactory observations have been made on swarm-spore formation. Here in some genera large and small swarmers (macro- and microspores) are formed by different individuals, and this has led to the inference that they may be macro- and microgametes although proof is lacking. In some species, *e. g.*, *Collosphæra huxleyi*, macrospores are formed in one hemisphere and microspores in the other hemisphere of the same central capsule. In this sub-order also, *Zoöxanthellæ*, or yellow cells, are common.

Family 1. **Sphærozoidæ**, Haeckel.—Great colony forms without latticed skeletons but frequently with isolated tangential spicules of silica (*Collozoum*, *Sphærozoum*).

Family 2. **Collosphæridæ**, Joh. Müller.—Forms in which each central capsule is enclosed in a single latticed silicious skeleton (*Collosphæra*).

Sub-order 3. **Collodaria,** Haeckel em. Brandt and Haecker.

These are solitary Peripylea with greatly developed extracapsular plasm and are usually confined to surface waters. They are usually spherical with no skeleton at all or with skeletons of the simplest type. They are uninucleate, with nuclei of complex structure.

Secondary nuclei are formed from chromatin (chromidia) which emerges from the giant nucleus into the endoplasm, the process being different for different forms described, until the entire substance of the original nucleus is exhausted. The secondary nuclei become the nuclei of flagellated swarmers. Despite the excellent work of Brandt, Moroff, Hartmann and Hammer, Huth, *et al.*, the reproduction of the much-studied species of *Thalassicolla* is still incomplete, and complications described by Haecker on *Oroscena regalis*, indicate that no common type is characteristic of the group.

Family 1. **Physematidæ**, Brandt.—Forms in which the capsular membrane is very thin, the nucleus globular and the endoplasm with characteristic vacuoles.

Family 2. **Thalassicollidæ**, Haeckel em. Brandt.—Forms similar to the above but without intracapsular vacuoles. In both families silicious spicules may be present or absent (*Thalassicolla*).

Family 3. **Thalassophysidæ**, Brandt.—Forms similar to the above but with the nucleus drawn out into peripheral pouches (*Thalassophysa*).

Family 4. **Thalassothamnidæ**, Haecker.—Huge forms with a single giant double spicule, and with globular or branched central capsule. (*Thalassothamnus*, Haecker, *Cytocladus*, Schröder).

Family 5. **Orosphæridæ**, Haeckel.—Forms with a thick latticed skeleton bearing radiating thorn-like and branching spines (*Oroscena*, Haeckel).

Order II. **ACTIPYLEA**, Hertwig.

In addition to the regularity in distribution of the pores of the central capsule, the Actipylea or Acantharia are distinguished from all other Radiolaria by the composition of the skeleton. Not only does it differ in its chemical make-up (strontium sulphate) but it also differs in its mode of formation and in its structure. The essential elements of the skeleton are radial bars which originate in the center of the central capsule and extend outward through the capsule and extracapsular protoplasm. These bars, furthermore, are arranged in a definite pattern which is so characteristic and invariable that it has become known as the Müllerian law. With a few exceptions in which the number of bars is a multiple of 20, the bars are twenty in number and the geometrical arrangement indicated by the Müllerian law, is such that the points of the spines fall in five circles parallel with the equator, and with four spines to each circle. The spines or bars, are named according to this scheme: Polar, tropical, equatorial, sub-tropical and sub-polar (Fig. 152).

The spines are covered with a sheath of gelatinous nature to which peculiar muscular threads—termed myophrisks—are attached.

With contraction of these threads, form and volume changes result in hydrostatic accommodations such that the organism rises or falls in the water.

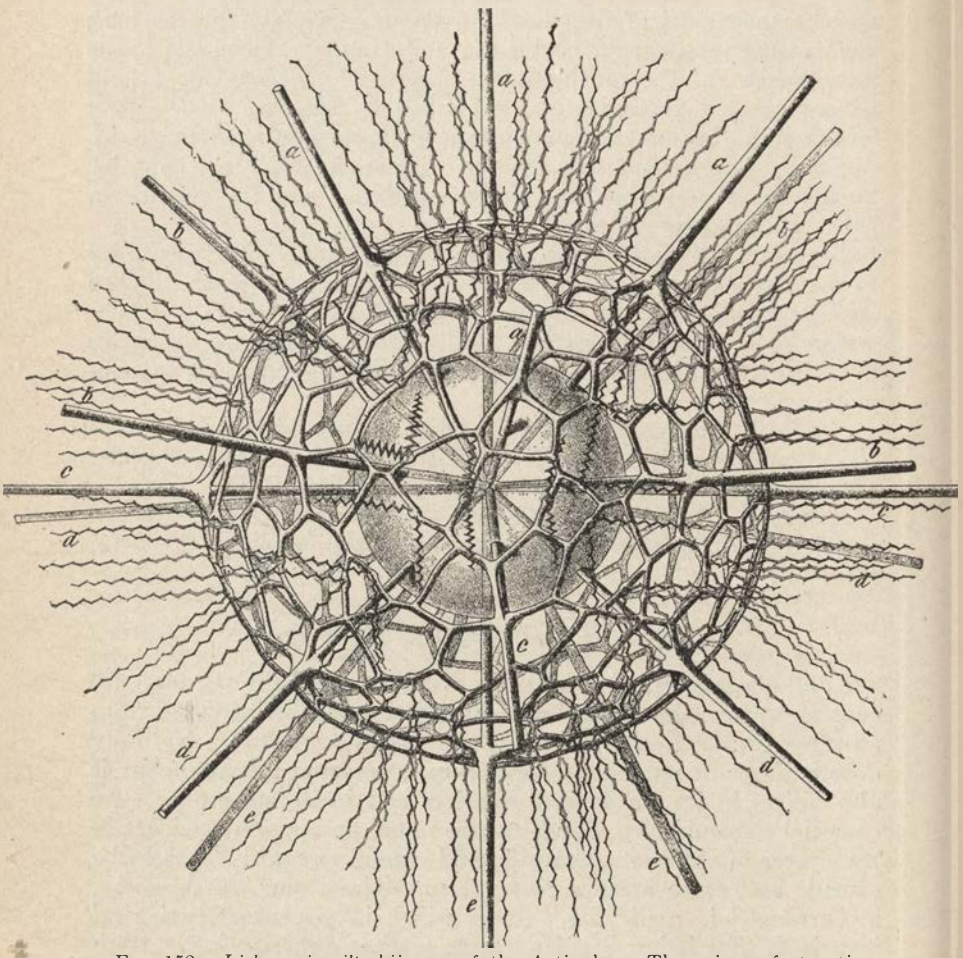

FIG. 152.—*Lichnaspis giltochii*, one of the Actipylea. The spines of strontium sulphate are arranged in accordance with the "Müllerian law" as follows: *a*, *a*, *a*, *a*, northern polar; *b*, *b*, *b*, *b*, northern tropical; *c*, *c*, *c*, equatorial; *d*, *d*, *d*, *d*, southern tropical; and *e*, *e*, *e*, southern polar. (From Calkins after Haeckel.)

In many cases the spines are bound together by a latticed skeleton consisting of twenty plates, each plate formed as an outgrowth from a spine. Other peculiarities of this Order are found in the thinness of the capsular membrane, frequent intracapsular yellow cells, and definite axopodia in addition to the usual rhizopodia.

Family 1. **Acantharidæ**, Haeckel.—Forms with 20 spines arranged according to the Müllerian law and all of approximately the same length (*Acanthometra elastica*).

Family 2. **Sphærophractidæ**, Haeckel.—Forms with 20 spines of equal length arranged as above; each spine with outgrowths from one or more places. These outgrowths are equally distant from the center and may fuse, forming one, two or more concentric shells or skeletons (*Sphærocapsa*, Haeckel).

Family 3. **Prunophractidæ**, Haeckel.—Forms similar to the above but with spines of unequal length resulting in correspondingly characteristic changes in the central capsule, skeleton, and axial relations. (*Thoracaspis*, Haeckel).

Family 4. **Actinellidæ**, Haeckel.—In this group the spines are more than 20 in number; Haeckel held that they are in multiples of 20, and he regarded this family as the most primitive of the Acantharia. (*Actinelius*, Haeckel, *Podactinelius*, Schröder, *Litholophus*, Haeckel).

Order III. **MONOPYLEA**, Hertwig.

The Radiolaria included in this Order are richly represented amongst fossil and recent types. They differ from all others in having a central capsule with its openings confined to one pole. The pores are in a portion of the central capsule membrane which forms the base of a conical volume of endoplasm, the podoconus, the apex of which is in the center of the endoplasm. The form of the central capsule is frequently fantastic, or radial, or bilaterally symmetrical. The nucleus is single, elongate and excentric in position.

The skeleton is invariably silicious and may be developed in a multitude of different forms based upon modifications of three parts, the basal spines, the "head" or capitulum, and the ring. These may be independent, or connected by fused spines, etc., with the tendency to form latticed skeletons which furnish the basis for the other name—Nassellaria—first used by Ehrenberg and amended by Haeckel to include forms in this Order.

Family 1. **Nassoidæ**, Haeckel.—Monopylea without skeletons.

Family 2. **Plectoidæ**, Haeckel.—Forms without capitulum or ring, but with basal spines which are frequently branched.

Family 3. **Stephoidæ**, Haeckel.—Invariably with a simple or multiple ring but without capitulum or basal spines.

Family 4. **Cyrtoidæ**, Haeckel.—With a well developed capitulum which is attached to the basal spines but without ring.

Family 5. **Spyroidæ**, Ehrenberg.—Here the well developed capitulum is divided by a sagittal cleft into two halves; basal spines also present.

Family 6. **Botryoidæ**, Haeckel.—Forms similar to those of the last family but without basal spines and with a mantle-like lengthening of the capitulum.

Order IV. **TRIPYLEA**, Hertwig.

In this great group of the Radiolaria all forms agree in having a central capsule with three openings. One of these, the astropyle, is the main or primary opening, the others, parapyles, are secondary. The primary opening is at the base of the main axis of the capsule and is covered by a radially-striped membrane the center of which is drawn out into a tube the opening of which is surrounded by a mass of secretory material and pigment—the pheodium. Hence Haeckel's name for the order the Pheodaria.

The bars of the silicious skeletons are hollow in the main, although solid parts may also occur.

Haecker divides the Tripylea into six groups of families which Doflein ranks as Legions but we designate them here as Sub-orders.

Sub-order 1. **Phæocystina**, Haeckel.

These are either naked forms or provided with a simple skeleton of numerous hollow bars which arise either independently in the protoplasm or are bound together to make a star-form group.

Family 1. **Aulacanthidæ**, Haeckel.—Here the skeleton consists of hollow radial spines and a mantle of very fine tangential tubes. (*Aulacantha scolymantha*, Haeckel.)

Family 2. **Astracanthidæ**, Haecker.—Forms with radially arranged hollow spines, the distal ends of which are variously developed while the proximal ends come from the outer surface of a central, hollow sphere. The central capsule here, is double.

Sub-order 2. **Phæosphæria**, Haeckel.

The skeletons of these forms consist of one, or two concentric shells; shell-openings if present, are limited to the inner shell.

Family 3. **Aulosphæridæ**, Haeckel.—Skeletons in the form of a simple latticed shell formed by hollow radial bars and a tangential meshwork; no shell opening present.

Family 4. **Cannosphæridæ**, Haeckel.—Here the skeletons are composed of two concentric shells bound together by radial bars; the inner shell has an opening (pylom).

Family 5. **Sagosphæridæ**, Haeckel.—With one or two concentric shells without shell openings; the bars of the meshwork are solid, thin and flexible.

Sub-order 3. **Phæocalpia**, Haeckel.

Shells monaxonic or polyedral with pylom or opening and with radial spines variously arranged.

Family 6. **Castanellidæ**, Haeckel.—Shells usually globular or monaxonic with uniformly distributed wide, rounded pores, and usually with numerous radial spines distributed on all sides.

Family 7. **Circoporidæ**, Haeckel.—Shells globular or polyedral, with a crown of pores at the base of the radial spines. The latter which are limited in number and have terminal branches, are usually arranged in geometrical patterns.

Family 8. **Tuscaroridæ**, Haeckel.—Shells usually flask-form with narrow pore canals and rather short unbranched radial spines grouped in one or two crowns about the main axis.

Family 9. **Porospathidæ**, Borgert.—Shells with papilliform excrescences or covered with a trigonal meshwork. Radial spines present on all sides.

Sub-order 4. **Phæogromia**, Haeckel.

Here the skeleton is usually bilaterally symmetrical with a shell mouth and definitely localized radial spines.

Family 10. **Challengeridæ**, J. Murray.—Shells with "diatom" structure; shell opening with a one-sided "peristome" formation. Radial spines if present only in the median plane more rarely grouped around the aboral pole.

Family 11. **Medusettidæ**, Haeckel.—Shells smooth or ornamented with small spines. Radial spines limited exclusively to the edge of the shell mouth

Sub-order 5. **Phæoconchiæ**, Haeckel em. Haecker.

Here the shells consist of two usually thick-walled valves which are perforated by rounded or slit-like pores.

Family 12. **Conchariidæ**, Haeckel.

Sub-order 6. **Phæodendria**, Haecker.

Shells consisting of two thin-walled valves each with a conical process from which originate diverging, branched tubes.

Family 13. **Cœlodendridæ**, Haeckel, em. Haecker.

KEY TO COMMON GENERA OF SARCODINA.

Class II. **RHIZOPODA**, von Sieb.

1. Naked; Heliozoa-like; radiating pseudopodia.....Sub-class 1. Proteomyxa
   Naked or shelled; pseudopodia not Heliozoa-like..... 2
2. With myxopodia and plasmodium formation.....Sub-class 2. Mycetozoa
   No plasmodium formation..... 3
3. With calcareous shells; marine Sub-class 3. Foraminifera
   Naked or with chitinous tests Sub-class 4. Amœbæa

SUB-CLASS 1. **PROTEOMYXA.**

1. Individuals Heliozoa-like; usually solitary. 2
Individuals fuse into thread-like plasmodia
Family 1. LABYRINTHULIDÆ
2. With flagellated swarmers.....Family 2. ZOÖSPORIDÆ
Without flagellated swarmers...Family 3. VAMPYRELLIDÆ

Family 1. **Labyrinthulidæ,** Hæck.

One genus only; little known.....Genus *Labyrinthula*

Family 2. **Zoösporidæ,** Zopf-Delage.

Intracellular parasites of Algæ, Volvolx, etc.
Genus *Pseudospora*
Starch-eating amœboid forms.....Genus *Protomonas*
Free-living, body red; marine.....Genus *Protomyxa*

Family 3. **Vampyrellidæ,** Doflein.

Form changeable; colorless; ray-like pseudopodia.....Genus *Nuclearia*
Body branched; naked; pseudopodia delicate
Genus *Arachnula*
Color reddish; ectoparasitic on Algæ..Genus *Vampyrella*
Color greenish; cysts of cellulose.....Genus *Chlamydomyxa*
Colonial forms.....Genus *Myxodictium*
Body sharply pointed at base of pseudopodia
Genus *Biomyxa*
Protoplasm of body and pseudopodia yellow
Genus *Rhizoplasma*
Body yellow; pseudopodia colorless...Genus *Dictomyxa*

SUB-CLASS II. **MYCETOZOA,** DE BARY.

Pseudoplasmodium in some; no peridia nor capillitia; sporangium a mere mass of spores.....Order 1. ACRASIDA
Parasitic; no peridia nor capillitia...Order 2. PHYTOMYXIDA
Plasmodia; peridia and capillitia....Order 3. EUPLASMODIDA

ORDER 1. **Acrasida,** VAN TIEGHEM.

Amœbæ solitary; stalked spore-case Family 1. SAPPINIIDÆ
Amœbæ grouped; sori from group..Family 2. GUTTULINIDÆ
Amœbæ grouped; stalks of sori hardened amœbæ.....Family 3. DICTYOSTELIDÆ

Family 1. **Sappiniidæ,** Dangeard.

One genus and species; dung of horse, cow, dog, etc.....Genus *Sappinia*

Family 2. **Guttulinidæ,** Cienk.

Cells do not form stalks of sori.....Genus *Copromyxa*
Short stalks bearing sori.....Genus *Guttulina*

Family 3. **Dictyostelidæ,** Rostafinsky.

1. Stalks unbranched..... 2
Stalks branched.....Genus *Polyspondylium*
2. Spores without definite arrangement Genus *Dictyostelium*
Spores in row like string of beads...Genus *Acrasis*

ORDER III. **Euplasmodida,** LISTER.

Spores exposed on surface of sporophores
Sub-order 1. EXOSPOREA
Spores in sporangia.....Sub-order 2. MYXOGASTRES

SUB-ORDER 1. **Exosporea**, ROSTAF.

One genus; spores exposed; no sporangia
Genus *Ceratiomyxa*

SUB-ORDER 2. **Myxogastres**, FRIES.

(Key to genera adapted from MacBride, 1922)
Spore mass black or violaceous, rarely ferruginous..... *Series A*
Spore mass never black; usually brown, yellow, etc..... *Series B*

Series A
With delicate thread-like capillitium; sporangia more or less calcareous.....Legion 1. PHYSARALES
With capillitium and columella; rarely calcareous.....Legion 2. STEMONITALES

Series B
Capillitium imperfect or none; spores brown, rarely purple.....Legion 3. CRIBARIALES
Capillitium of interwoven plates or tubules; spores pale or ashen.....Legion 4. LYCOGALALES
Capillitium of sculptured threads; spores yellow.....Legion 5. TRICHIALES

Legion 1. PHYSARALES MacBr.
Fructification often calcareous throughout; capillitium intricate.....Family 1. PHYSARIDÆ
Lime in peridium only, or also in stipe; capillitium simple.....Family 2. DIDYMIIDÆ

Family 1. **Physaridæ**, MacBr. em.
1. Fructification an æthalium.....Genus *Fuligo*
Fructification an aggregate of sporangia... 2
2. Peridium calcareous..... 3
Peridium apparently limeless, at least outside..... 6
3. Capillitium calcareous throughout..Genus *Badhamia*
Capillitium largely hyaline..... 4
4. Sporangia globose; dehiscence irregular
Genus *Physarum*
Sporangia vasiform or tubular..... 5
5. Dehiscence by lid-covered opening..Genus *Craterium*
Dehiscence irregular; peridium inverted
Genus *Physarella*
6. Sporangia sessile with irregular outlines
Genus *Cienkowskia*
Sporangia distinct.....Genus *Leocarpus*

Family 2. **Didymiidæ**, MacBr. em.
1. Fructification an æthalium.....Genus *Mucilago*
Fructification not an æthalium..... 2
2. Peridium single..... 3
Peridium double; outer one gelatinous.... 4
3. Calcareous deposits crystalline; stellate
Genus *Didymium*
Calcareous deposits in form of scattered scales.....Genus *Lepidoderma*
4. Outermost peridium gelatinous.....Genus *Colloderma*
Outer peridium hardened.....Genus *Diderma*

Legion 2. Stemonitales MacBr.
1. Fructification æthalium-like; columella rudimentary or absent.....Family 1. Amourochætidæ
Fructification with distinct sporangia..... 2
2. Capillitium well-defined; columella prominent, long.....Family 2. Stemonitidæ
Capillitium developed from top of columella.....Family 3. Lamprodermidæ

Family 1. **Amourochætidæ,** MacBr. em.
A single genus.....Genus *Amourochæta*

Family 2. **Stemonitidæ,** MacBr. em.
1. Sporangia grouped; capillitium with vesicles.....Genus *Brefeldia*
Sporangia distinct..... 2
2. Stipe and columella jet black..... 3
Stipe and columella whitish; calcareous Genus *Diachæa*
3. Tips of capillitium branches free....Genus *Comatricha*
Tips united forming a surface network Genus *Stemonitis*

Family 3. **Lamprodermidæ,** MacBr. em.
1. Columella through sporangium, capillitium apical.....Genus *Enerthenema*
Columella only part way through sporangium..... 2
2. Capillitium fully developed..... 3
Capillitium rudimentary; minute forms Genus *Echinostellium*
3. Capillitium does not form a net....Genus *Clastoderma*
Capillitium forms an intricate net..Genus *Lamproderma*

Legion 3. Cribrariales MacBr.
1. Sporangia distinct and separated..... 2
Sporangia associated..... 3
2. Walls of sporangia perforate, especially above.....Family 1. Cribrariidæ
Walls not perforated; sporangia with lid Family 2. Orcadellidæ
3. Sporangia irregularly grouped in delicate membrane.....Family 3. Liceidæ
Sporangia definitely grouped..... 4
4. Walls of sporangia not perforated; tubular Family 4. Tubiferidæ
Walls of sporangium perforated or frayed Family 5. Reticulariidæ

Family 1. **Cribrariidæ,** MacBr. em.
Peridium with meridional ribs or thickenings Genus *Dictydium*
Peridium with apical thickenings only.Genus *Cribraria*

Family 2. **Orcadellidæ,** MacBr. em.
A single genus.....Genus *Orcadella*

Family 3. **Liceidæ,** MacBr.
A single genus.....Genus *Licea*

Family 4. **Tubiferidæ,** MacBr. em.
1. Sporangia stipitate; clustered.....Genus *Alwisia*
Sporangia in linear series..... 2
2. Spores olivaceous.....Genus *Lindbladia*
Spores umber.....Genus *Tubifera*

Family 5. **Reticulariidæ,** MacBr. em.
1. Spores brownish or umber..... 2
Spores yellowish.....Genus *Dictydiæthalium*
2. Sporangia bounded by broad perforated plates.....Genus *Enteridium*
Sporangia wholly indeterminate....Genus *Reticularia*

Legion 4. Lycogalales MacBr.
One genus only.....Genus *Lycogala*

Legion 5. Trichiales MacBr.
1. Capillitium a distinct net; no spiral bands
Family 1. Arcyriidæ
Capillitium threads fixed or free; no net... 2
2. Capillitium threads free; with spiral bands
Family 2. Trichiidæ
Capillitium threads attached..... 3
3. Threads attached at both ends..... 4
Threads attached at one end if at all
Family 3. Perichænidæ
4. Threads plain or slightly roughened
Family 4. Dianemidæ
Threads definitely sculptured...Family 5. Prototrichiidæ

Family 1. **Arcyriidæ,** MacBr. em.
1. Capillitium elastic..... 2
Capillitium non-elastic.....Genus *Lachnobolus*
2. Capillitium attached at base; no hamate branches.....Genus *Arcyria*
Capillitium centrally attached, with hamate branches.....Genus *Heterotrichia*

Family 2. **Trichiidæ,** MacBr. em.
1. Capillitium threads long, centrally attached 2
Capillitium threads short, free, sometimes branched..... 3
2. Sculpture spiral.....Genus *Hemitrichia*
Sculpture reticulate.....Genus *Calonema*
3. Threads, elaters, marked by spiral bands
Genus *Trichia*
Threads with irregular sculpture or none
Genus *Oligonema*

Family 3. **Perichænidæ,** MacBr. em.
Sporangia more or less grouped; dehiscence irregular.....Genus *Ophiotheca*
Sporangia grouped, polygonal; dehiscence by lid.....Genus *Perichæna*

Family 4. **Dianemidæ,** MacBr. em.
Capillitium threads attached at one end only or free.....Genus *Margarita*
Capillitium threads attached at each end
Genus *Dianema*

Family 5. **Prototrichiidæ,** MacBr. em.
A single genus.....Genus *Prototrichia*

Sub-class III. **FORAMINIFERA,** D'Orb.

Doflein includes all of the testate rhizopods as a subdivision—Monothalamia—of the Foraminifera. Such a system does not

take into consideration such widely different types of pseudopodia as the filopodia and myxopodia, and relationship to the many-chambered Foraminifera in other respects seems equally remote. There are some types such as *Lieberkühnia*, which approach the foraminiferon plan of structure and their systematic position must remain somewhat of a puzzle, but for the present we shall retain the older plan of grouping them with the testate Amœbæa. Similarly it is a question whether shell-less forms with myxopodia should be included as Reticulosa with the Foraminifera under the term Nuda as Rhumbler does, or be grouped with fresh-water types in the subdivision Proteomyxa. The latter course is followed in the present work and the Foraminifera are limited to the shell-forming types of marine Sarcodina having myxopodia. This method of solving the difficulty has the one advantage of grouping together all of the monothalamous forms of Sarcodina and of strictly limiting the Foraminifera to polythalamous types. It is a matter, frankly, of expediency and does not involve any one of the numerous theories of rhizopod phylogeny.

The tendency has been toward a simplification of the classification of Foraminifera. Carpenter's division into Perforata and Imperforata, also adopted by Brady involves the problem of isomorphs and questionable affinities. Lister's classification, in which the group is divided into some thirty-two families, seems unnecessarily cut up. Rhumbler's system is much more simple and is adopted here. He distributes the ten families recognized as Foraminifera amongst five "Family Groups" which we indicate as orders in the following system.

Order I. **Archi-Monothalamida = Archi-Monothalmidia,** Rhumbler.

Shells primarily of a single chamber yet often with incomplete septa, incompletely dividing the single chamber into many chambers.

Family 1. **Rhabdamminidæ,** Rhumbler—Shells gelatinous, pseudochitinous or with foreign bodies cemented on a chitinous membrane; always one-chambered but of variable (globular, tube-like, asteroid) form. The chamber of the shell may be partially broken up by incomplete septa; pores absent or casual; openings one or many.

Genera: *Myxotheca,* Schaudinn; *Allogromia,* Rhumbler (both salt and fresh water); *Dendrotuba,* Rhumbler; *Astrorhiza,* Sandahl; *Saccammina,* M. Sars; *Psammosphæra,* F. E. Schultze; *Rhizammina,* Brady; *Hyperammina,* Brady; *Rhabdammina,* M. Sars; *Haliphysema,* Bowerbk.; *Hippocrepina,* Parker.

Family 2. **Ammodisculinidæ**, Rhumbler.—In part sand in part lime shells of tubular, discoid or rolled form, imperforate or perforate.

Genera: *A.* Imperforate sand shells. Genus *Girvanella*, Nich. and Eth.; *Lituotuba*, Rhumbler; *Psammonyx*, Doderlein; *Ammonodiscus*, Reuss.; *B.* Imperforate lime shells. *Cornuspira*, M. Schultze; *C.* Perforate lime shells, with chamber-like swellings and incomplete septa.

Genera: *Spirillina*, Bory; *Tetrataxis*, Ehren.; *Patellina*, Williamson.

Order II. **Nodosalida = Nodosalidia**, Rhumbler.

Shells usually chambered but monothalamous forms may arise by separation of newly-formed chambers. Chambers in straight or slightly curved rows, occasionally in a flat spiral row (*Cristellaria*); initial chamber without a bent neck.

Family 3. **Nodosamminidæ**, Rhumbler.—Shells sandy or more or less calcareous; perforate or imperforate; invariably polythalamous; chambers in a straight or slightly bent row.

Genera: *Nodosinella*, Brady; *Rheophax*, Montf.

Family 4. **Nodosariidæ**, Rhumbler.—Shells pure limestone with many fine and closely set perforations.

Genera: *Nodosaria*, Lamarck; *Vaginulina*, Fornas; *Lagena*, Walker and Boys; *Cristellaria*, Link.

Order III. **Flexostylida = Flexostylidia**, Rhumbler.

The globular initial chamber is provided with a flexible, sac-like or tubular "neck" which winds around the original chamber and becomes attached to the second chamber. Walls usually imperforate or with sparse perforations; shells usually calcareous, porcellanous and not infrequently mixed with sand. In brackish water shells pseudochitinous or with sand crystals, in deep sea also, but exceptionally silicious.

Family 5. **Miliolinidæ**, Brady em. Rhumbler.—Shells spirally wound without cyclical growth; imperforate except for the initial chamber of *Peneroplis* which is perforate.

Genera: Group *A:* Chambers long, usually including one-half a spiral turn. *Nodobecularia*, Rhumbler; *Nubecularia*, Defr.; *Calcituba*, Roboz; *Biloculina*, d'Orb.; *Miliolina*, Williamson; *Triloculina*, D'Orb; *Quinqueloculina*, d'Orb.; *Ophthalmidium*, Kubl. and Zwingli; *Spiroloculina*, d'Orb. Group *B:* Chambers very short so that many are included in one turn, but occasionally very wide; *Peneroplis*, Montf.; *Alveolina*, d'Orb.

Family 6. **Orbitolinidæ**, Reuss em. Rhumbler.—In the later rows chamber growth becomes cyclical or ring form, with division of

the chambers into subchambers; a single mouth present only in the initial chambers; in later chambers a large number of so-called mouth-pores are present.

Genera: Group *A:* Calcareous; imperforate. *Archiacina*, Meun.-Chalm.; *Orbitolites*, Link.; *Orbiculina*, Lam. Group *B:* Calcareous; perforate. *Orbitoides*, d'Orb. (fossil only).

Order IV. **Textulinida** = **Textulinidia**, Rhumbler.

Polythalamous with initial chamber without neck and succeeding chambers arranged in two or more alternating rows which, in more complex forms, are spirally wound. The shell material is lime, or lime with sand, or sand, and the walls are usually perforate, rarely imperforate.

Family 7. **Textulinidæ**, Rhumbler.—With the characters of the group.

Genera: *Textularia*, Defr.; *Cribrostomum*, Moeller; *Bolivina*, Orb.; *Verneuilina*, Orb. (with three rows); and *Cassidulina*, Orb.

Order V. **Rotalida**, Brady em. Rhumbler = **Rotaliaridia**.

Polythalamous with initial chamber without neck, the succeeding chambers forming a flat spiral or with a portion developed as a turbo-spiral. In some cases with an irregular heap of terminal chambers (acerval).

Family 8. **Trochamminidæ**, Rhumbler.—Shell material sand, sand and lime or pure lime; no dimension of single chambers relatively long.

Genera: *Endothyra* (Carboniferous), Philips; *Haplophragmium*, Reuss; *Trochammina*, Jon. and Park.; *Carterina*, Brady; (here the shell is made up of calcareous plates cemented together in the form of a mosaic); *Cyclammina*, Brady.

Family 9. **Fusilinidæ**, Rhumbler.—Shells imperforate, with chambers much elongate in the direction of the turns and divided into numerous subchambers. Only fossil forms known. *Fusulina*, Fischer; *Schwagerina*, Moeller; *Schellwienia*, Staff and Wedek.

Family 10. **Rotaliidæ**, Brady em. Rhumbler.—Calcareous and invariably perforate. Spirals are so wound that either all chambers are visible on the upper surface but only the last chamber from the under side, or only the last chamber is visible from either side. The more complex forms are provided with a canal system.

Genera: *Truncatulina*, Orb.; *Pulvinulina*, Jon. and Park.; *Rotalia*, Lamarck; *Discorbina*, J. and P.; *Polytrema*, Risso; *Tinoporus*, Montf. The Globigerina group includes pelagic or floating forms with swollen chambers which start with spiral arrangement but become irregular; usually

with bristles or ridges, etc., apparently useful in flotation. *Globigerina,* Orb. (the last greatly swollen chamber includes within it all of the earlier chambers); *Hastigerina,* W. Thomp.; The Nummulites group includes more complicated types with canal system: Here: *Nonionina,* d'Orb.; *Polysiomella,* Link; *Operculina,* d'Orb.; *Nummulites,* Link; and *Amphistigina,* d'Orb.

SUB-CLASS IV. **AMŒBÆA,** BÜTSCHLI.

Naked forms; pseudopodia lobopodia or lamellipodia.....Order 1. AMŒBIDA
Testate or membraned forms.....Order 2. TESTACEA

ORDER 1. **Amœbida,** AUT.

1. Diphasic forms, amœboid and flagellated stages.....Family 1. BISTADIIDÆ
Monophasic forms, amœboid only..... 2
2. Free-living; water, earth, moss, etc..... 3
Parasites of cavities and tissues..Family 2. ENDAMŒBIDÆ
3. Without cytoplasmic "Nebenkern" Family 3. AMŒBIDÆ
With cytoplasmic "Nebenkern".Family 4. PARAMŒBIDÆ

Family 1. **Bistadiidæ,** Doflein.

Two flagella in flagellated phase.....Genus *Nägleria*
One flagellum in flagellated phase.....Genus *Craigia*
Three flagella in flagellated phase.....Genus *Trimastigamœba*

Family 2. **Endamœbidæ.**

1. Vegetative forms with one nucleus..... 2
Vegetative forms with two nuclei...Genus *Dientamœba*
2. Encysted stage with huge glycogen mass Genus *Iodamœba* (*Endolimax*)
Encysted stage without large glycogen mass 3
3. Reproduction by budding in cells with eight nuclei.....Genus *Councilmania* (?)
Reproduction by division.....Genus *Endamœba*

Family 3. **Amœbidæ,** Doflein.

1. Actively moving forms with lobose pseudopodia..... 2
Sluggish forms; no definite pseudopodia Genus *Pelomyxa*
2. Large forms, several pseudopodia...Genus *Amœba*
Small forms moving as one pseudopodium Genus *Vahlkampfia*

Family 4. **Paramœbidæ,** Doflein.

Free living or parasitic, one genus.....Genus *Paramœba*

ORDER 2. **Testacea,** DEL. and HER.

1. Tests simple; membranous, plastic or rigid 2
Tests rigid, with foreign bodies, plates or scales..... 3
2. Pseudopodia lobose or simply branched Family 1. ARCELLIDÆ
Pseudopodia reticulate, forming a network Family 4. GROMIIDÆ

3. Chitinous test covered by foreign bodies
Family 2. DIFFLUGIIDÆ
Chitinous test with plates made by organism.....Family 3. EUGLYPHIDÆ

Family 1. **Arcellidæ.**

1. Tests membranous and flexible..... 2
Tests membranous; rigid; with or without foreign bodies..... 9
2. Test like inverted watch-glass; aperture full diameter..... 3
Test cup-like or sac-like..... 4
3. Test with hyaline margin (Fig. 153.) Genus *Pseudochlamys*
Tests completely hyaline.....Genus *Pyxidicula*
4. Tests cup-like..... 5
Test bag or sac-like..... 7

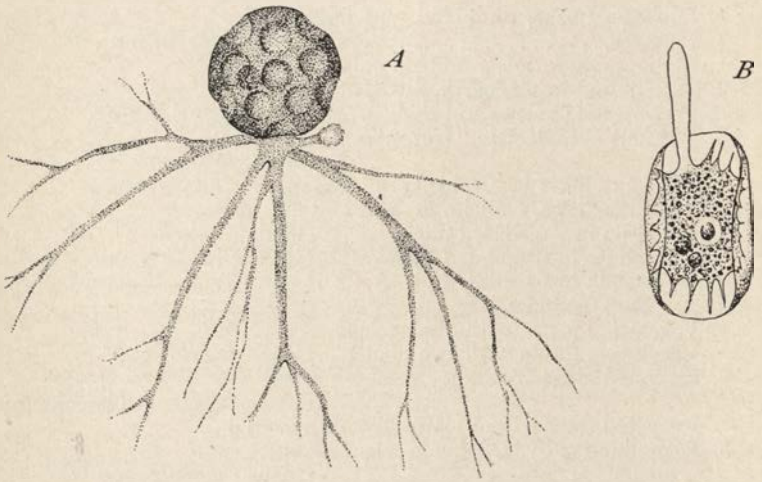

FIG. 153.—*A*, *Hyalosphenia*? sp. (Original,) *B*, *Pseudochlamys patella* after Clap and Lachm.

5. Margin of test aperture turned in..... 6
Test aperture with diaphragm-like membrane.....Genus *Diplochlamys*
6. Cell body uninucleate (*Rhogostoma*) Genus *Amphizonella*
Body with more than one nucleus..Genus *Zonomyxa*
7. Crown of test with circular and radial ridges.....Genus *Microcorycia*
Crown of test simple; aperture an elastic slit..... 8
8. Test non-encrusted ovoid sac; aperture linear.....Genus *Capsellina*
Test sac-like, covered with foreign bodies Genus *Parmulina*
9. Test rigid; chitinous; without foreign bodies..... 10
Test more or less plastic; one or more pores Genus *Cochliopodium*

10. Test symmetrical..... 11
    Test asymmetrical (one species encrusted)
    Genus *Lesquereusia*
11. Tests circular in cross-section..... 12
    Tests ellipsoidal in cross-section..... 15
12. Free living forms..... 13
    Parasitic form (Fig. 154).....Genus *Chlamydophrys*
13. Pseudopodia lobose..... 14
    Pseudopodia short lobose with aciculate tip
    Genus *Difflugiella*

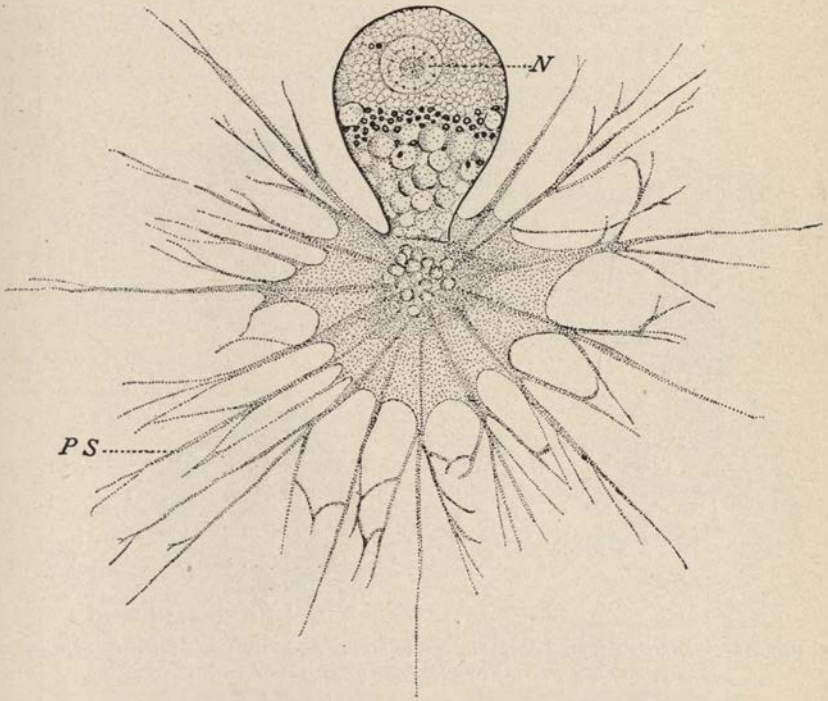

FIG. 154.—*Chlamydophrys stercorea.* (From Doflein after Schaudinn.)

14. Aperture of test with inturned margin
    Genus *Arcella*
    Margin not inturned; one lobose pseudopodium.....Genus *Leptochlamys*
15. Tests yellow or brown; minute; without pits .....Genus *Cryptodifflugia*
    Tests hyaline; transparent; usually with pits.....Genus *Hyalosphenia*

Family 2. **Difflugiidæ.**

1. Test circular in cross-section..... 2
   Test ellipsoidal in cross-section (compressed)..... 6

2. Aperture of test circular..... 3
   Aperture of test ellipsoidal or linear Genus *Plagiopyxis*
3. Aperture without lobed external collar.... 4
   Aperture with three or four lobed external collar.....Genus *Cucurbitella*
4. Aperture excentric in position.....Genus *Centropyxis*
   Aperture central; symmetrical..... 5
5. Test covered with diatom shells; pseudopodia pointed.....Genus *Phryganella*
   Test covered with sand, mud, detritus, etc. (Fig. 155).....Genus *Difflugia*

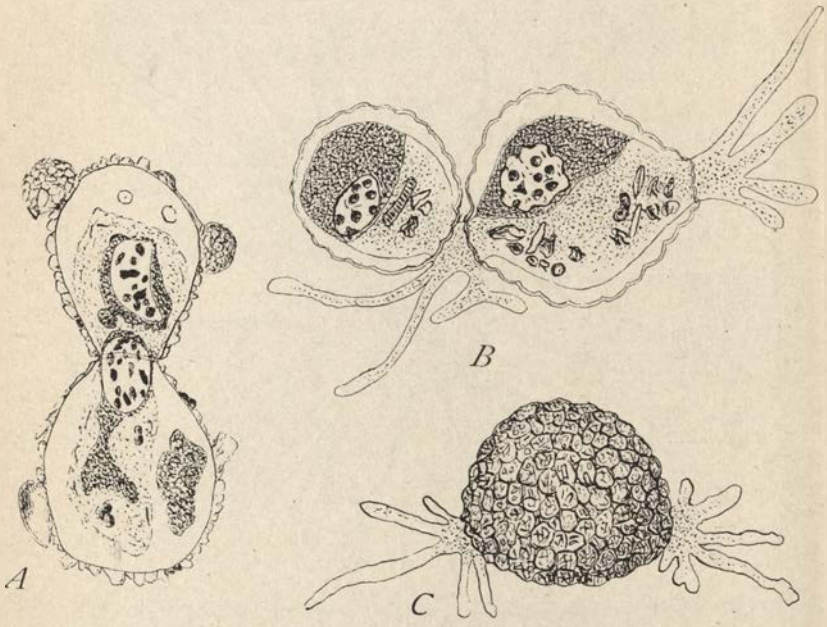

FIG. 155.—*Difflugia lobostoma* plastogamic stages formerly interpreted as evidence of conjugation. (From Calkins after Rhumbler.)

6. Test with constricted neck and internal shelf.....Genus *Pontigulasia*
   Test without internal shelf..... 7
7. Test with foreign particles on dome only.. 8
   Test covered; aperture a long and narrow slit.....Genus *Bullinula*
8. Aperture of test convex.....Genus *Heleopera*
   Aperture small, ellipsoidal, with thickened margins.....Genus *Awerintzia*

Family 3. **Euglyphidæ.**

1. Cells without chromatophores..... 2
   Cells with one or two blue-green chromatophores.....Genus *Paulinella*
2. Test curved, retort-shape..... 3
   Test dome-shape; not curved..... 5

3. Aperture terminal, oblique 4
   Aperture terminal not oblique . . . . . Genus *Nadinella*
4. Test with regular, small, plates; no membrane . . . . . Genus *Cyphoderia*
   Test with amorphous plates; aperture with membrane . . . . . Genus *Campascus*
5. Test circular in cross-section 6
   Test ellipsoidal in cross-section (compressed) 7
6. Dome with single long spine; plates fine
   Genus *Pareuglypha*
   Dome without spine . . . . . Genus *Euglypha*
7. Test asymmetrical 8
   Test symmetrical 9
8. Aperture circular; oblique; invaginated
   Genus *Trinema*
   Aperture oval; oblique; not invaginated
   Genus *Corythion*
9. Test plates circular or oval 10
   Test plates rectangular . . . . . Genus *Quadrula*
10. Test hyaline; transparent; plates numerous 11
    Test brown or colorless; aperture oval
    Genus *Assulina*
11. Aperture as in *Difflugia*, circular . . . . Genus *Nebela*
    Aperture linear with undulate border
    Genus *Placocista*

Family 4. **Gromiidæ.**

1. With one test aperture 2
   With two or more test apertures
   Sub-family 3. Amphistominæ
2. Filose pseudopodia directly from plasm
   Sub-family 1. Pseudogromiinæ
   Reticulate pseudopodia from peduncle
   Sub-family 2. Allogromiinæ

Sub-family 1. *Pseudogromiinæ*, Cash

1. Test of one piece 2
   Test bivalved . . . . . Genus *Clypeolina*
2. Test smooth; no foreign particles . . . Genus *Lecythium*
   Test covered with foreign particles 3
3. Test ovoid; no hair-like cirri . . . . . Genus *Pseudodifflugia*
   Test ovoid; flexible; with hair-like cirri
   Genus *Diaphoropodon*

Sub-family 2. *Allogromiinæ*, Rhumbler

1. Test ovoid; plastic, aperture lateral . Genus *Lieberkühnia*
   Test rigid or plastic; aperture terminal 2
2. Test oval or pyriform; not encrusted 3
   Test cylindrical; encrusted with foreign bodies . . . . . Genus *Rhynchogromia*
3. Test and organism minute; often colonial
   Genus *Microgromia*
   Test large, oval, solitary . . . . . Genus *Allogromia*

Sub-family. *Amphistominæ*, Cash

1. Test with two apertures 2
   Test with from three to six apertures
   Genus *Microcometes*

2. Test minute; hyaline; spheroidal; colored globule.....Genus *Diplophrys*
Test medium; oval; encrusted or not; with symbionts.....Genus *Amphitrema*

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CHAPTER VIII.

SPECIAL MORPHOLOGY AND TAXONOMY OF THE INFUSORIA.

Since the first discovery of *Vorticella* and allied forms of Protozoa by Leeuwenhoek in 1675, the Infusoria have been among the most favored of living things studied through the microscope. The designation Animalculæ, given to include all forms of microscopic life was changed by Ledenmüller to Infusoria in 1760–1763, and the entire phylum of Protozoa were included under this term by the majority of writers down to Bütschli in 1882. Dujardin, 1841, divided the "Infusoires" into rhizopods, flagellates and ciliates, a classification adopted by Bütschli who, however, limited the use of the term Infusoria to Protozoa bearing cilia at some period of the life history. Two classes are universally recognized today, the Ciliata with permanent cilia, and Suctoria with cilia in the embryonic phases only. The classification of the Infusoria approaches more closely to an ideal natural system than is possible at the present time with any other group of Protozoa.

The great majority of Infusoria are free-swimming but practically all Suctoria and several minor groups of the Ciliata are attached, while a few are parasitic. The majority of attached forms tend to radial symmetry; free-swimming types show the greatest variety of forms which in many cases may be traced to the effects of mode of life, but the fantastic shapes of sapropelic and of many parasitic types are difficult to reconcile with environmental conditions. The ideal generalized form of Ciliata is a spherical or ellipsoidal organism with the mouth at one end, contractile vacuole near the other, and lines of cilia starting from the mouth and running in longitudinal rows down the body. Shifting of the mouth with distortion of the lines of cilia, leads to various modifications of the generalized type which is most closely represented by *Holophrya* or *Prorodon* species (Fig. 166). A ventral surface bearing the mouth is established in the Hypotrichida which includes some of the most highly specialized forms of Protozoa.

The endoplasm is finely alveolar and much more fluid than the more highly differentiated cortex or ectoplasm. The endoplasm reveals different types of refringent granules during life, some of which have been identified as excretory granules (Prowazek, Niren-

stein), others as mitochondria (Fauré-Fremiet, Cowdry) and others as belonging to the Golgi apparatus (Nassonov). In addition to these, reserves of food substances, kinetic elements, and metaplastids of different kinds, with the nuclei make-up the substance of the endoplasm.

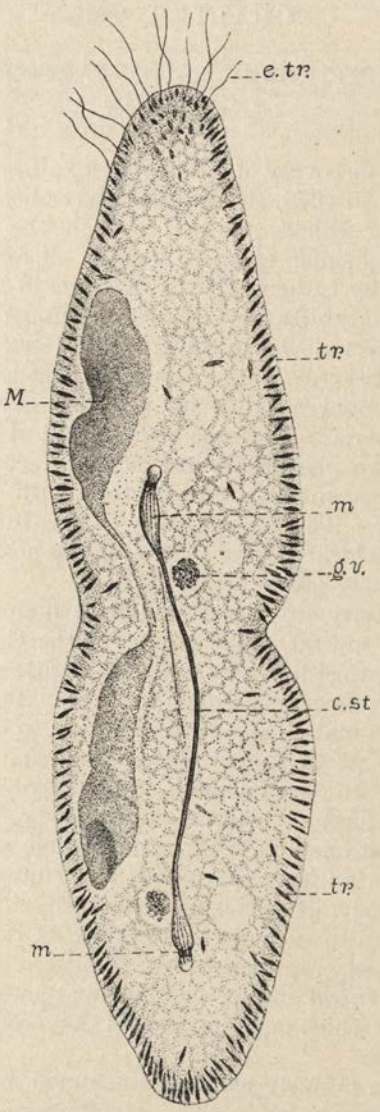

FIG. 156.—*Paramecium caudatum*. Section of a dividing individual. *c.st.*, connecting strand of dividing micronuclei; *e.tr.*, extruded trichocysts; *g.v.*, gastric vacuole; *M*, dividing macronucleus; *m*, *m*, divided micronuclei; *tr.*, trichocysts. (Original.)

Metaplastids are numerous and widely distributed. Of these trichites, trichocysts and "pharyngeal baskets" are the most characteristic. Trichites are elongate slender rods usually surrounding the mouth in gymnostomes and are generally interpreted as organs of support or protection. They are not limited to the oral region, however, and in some forms provide a protective cuirass about the posterior region (*Strombidium*). The oral trichites are numerous and closely applied and in some cases form a continuous and smooth tube extending deep in the endoplasm (some *Nassulas*, *Orthodon*, etc.). Trichocysts are shorter and more conspicuous; formed in the endoplasm they assume a tangential position in the cortex and may cover the entire surface (*Paramecium*, Fig. 156, *Frontonia*, etc.) or may be limited to certain regions (*Dileptus* proboscis, Fig. 157)). In a moving *Actinobolus* they are arranged as in *Paramecium*, but in a quiescent individual each trichocyst is carried out at the end of a long tentacle which this interesting ciliate has the power to protrude for feeding purposes (Fig. 81, p. 154).

The function of the trichocysts is still in dispute (Visscher, 1923). The substance of a trichocyst may be shot out in the form of a long thread which hardens on contact with water. In such forms, represented by *Paramecium*, *Frontonia* and other related forms, there appears to be no toxic action connected with the trichocysts, the threads affording protection by the formation of a net-like weft about the organism. In other cases, however, there is considerable evidence of toxic action and in such types the long threads are not formed. Visscher (1923) has described such toxic action on the part of the trichocysts of *Dileptus*, and the sudden paralysis

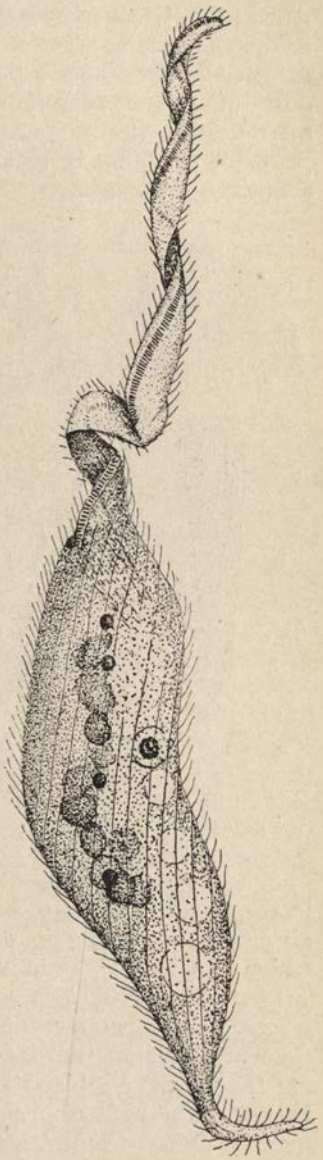

FIG. 157.—*Dileptus gigas*, with beaded macronucleus and twisted proboscis. (Original.)

of *Halteria grandinella* upon coming in contact with a tentacle of *Actinobolus* is interpreted as due to the toxic action of the minute trichocyst at the extremity of the tentacle (Calkins, Moody). In *Didinium nasutum* there is a zone of rods quite independent of the pharyngeal trichites and interpreted as trichocysts near the extremity of the seizing organ of this voracious animal (Fig. 89, p. 180). A *Paramecium* jabbed by this proboscis in one of the vigorous darts of *Didinium* is immediately paralyzed and the poisoning is attributed to the trichocyst material. While this interpretation is plausible it cannot be regarded as proved, and it must be admitted that the protoplasm itself may carry the toxic substance. Thus

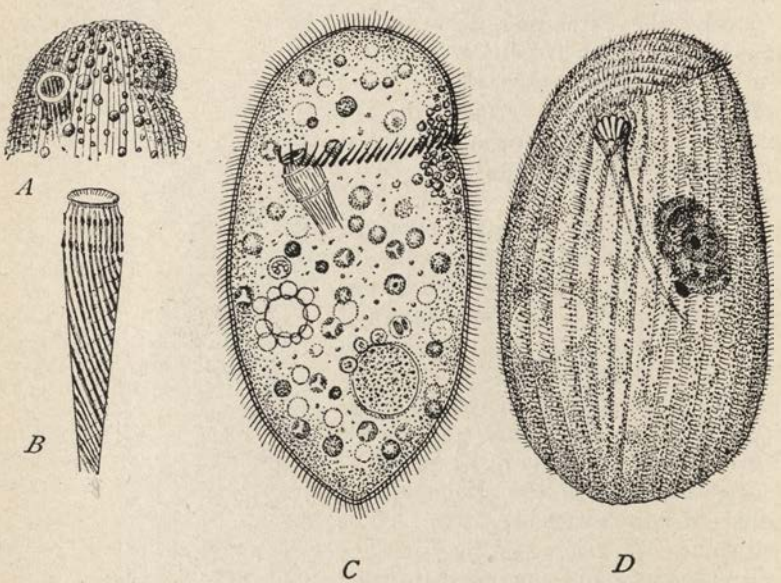

FIG. 158.—*Nassula aurea* (*C*) and details of basket (*A*, *B*, after Bütschli); *D*, *Chilodon* sp. (Original.)

in the Suctoria a ciliate or other small organism, is similarly paralyzed upon coming in contact with an outstretched tentacle in which no trichocysts can be demonstrated.

Pharyngeal baskets are characteristic of the Chlamydodontidæ where they form conspicuous oral armatures (Fig. 158). The elements forming the basket are much larger than trichites and are frequently combined in such a manner as to justify the term basket. The rods are usually constant in number in a species and may be united to form a tube at the posterior end of the basket, or in some cases may be united throughout. In Chilodon the basket is protrusible and serves a useful purpose in food-getting.

According to MacDougall (1925) the basket is dissolved in artificial gastric juice (pepsin) indicating a protein composition.

Metaplastic substances frequently appear in the form of pigments which impart a characteristic color to a species. These are probably connected with food metabolism and disappear in the absence of appropriate food materials. Thus the blue pigment "stentorin" of *Stentor cæruleus*, or *Folliculina* or the lavender of *Blepharisma undulans*, the red of *Mesodinium rubrum*, the black spot of *Tillina magna*, etc., are coloring matters of this type. Fats and oils also are frequent inclusions and when brilliantly colored, as in *Nassula aurea*, give a striking and a pleasing picture as the organism rolls through the water.

Chromatophores, as differentiations of ciliate protoplasm are not generally credited although Engelmann maintained that chlorophyll (?) occurs in *Vorticella campanulata*. Symbionts, however, are of frequent occurrence and give to *Paramecium bursaria*, *Stentor viridis*, *Ophrydium versatile* and some *Vorticella* species a bright green color.

Contractile vacuoles are practically universal amongst ciliates and Suctoria. Held in place in the denser cortex they never move about with cyclosis. They empty to the outside through a covered but thinned orifice spot in the cortex, the covering being liquefied at systole (Taylor, 1923). The vacuole system includes canals and reservoirs reaching a high degree of specialization in some forms, and ciliated excretory canals are said to be present in a few parasitic types (*Pycnothrix*, Schubotz, 1908).

The Infusoria are unique in having an almost universal nuclear apparatus in the form of dimorphic nuclei, macronucleus and micronucleus. Of these the macronucleus is large and usually homogeneous in structure (granular) and is highly variable in shape in different species. In some forms it is multiple and formed by repeated division of an original single nucleus (*Uroleptus mobilis*); in other cases attempted division results in a chain of nuclei connected by a common nuclear membrane giving rise to "beaded" nuclei (*Stentor, Spirostomum ambiguum, Uronychia transfuga*, etc.). It is frequently rod-shape as in *Diplodinium* (Fig. 2, p. 20), or horseshoe shape as in *Vorticella*, or very much branched as in *Dendrosoma, Ephelota* and other Suctoria (Fig. 159).

Micronuclei are minute and are usually partially embedded in the substance of the macronucleus. There is but little variation in form of the micronucleus in different species, but there is a great variation in the number present. In *Paramecium caudatum* and *P. bursaria* there is but one, while in *P. aurelia* and *P. calkinsi* there are two, and two are characteristic of the Pleurotrichidæ. The number of micronuclei runs up to eighty or ninety in *Stentor* and the number is intermediate in several other genera.

Macronuclei are generally regarded as "somatic" nuclei with an important part to play in general metabolism. They disappear by absorption and are replaced by products of micronuclear division at periods of reorganization by "endomixis," or by products of amphinuclei after conjugation. Chromosome formation, with a definite number of chromosomes, has been made out for a number of species of ciliates, but no definite chromosomes have been described from macronuclei. Evidence is accumulating to indicate that the micronucleus is the essential element of the cell in conjugation but other evidence is at hand to show that it is not essential for continued

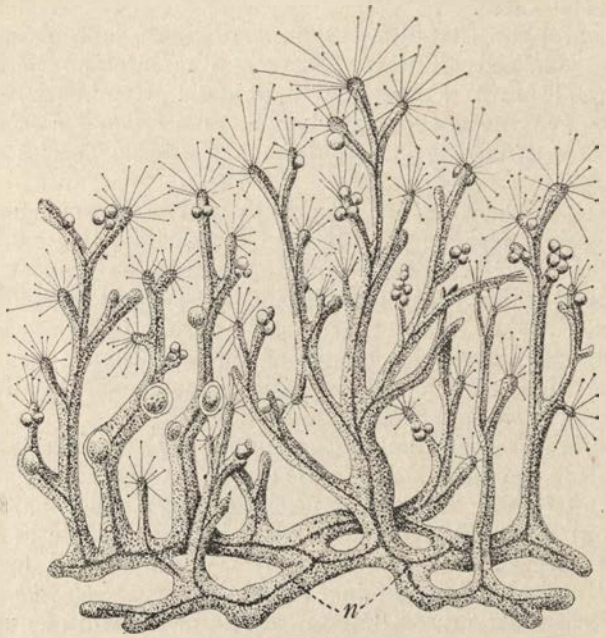

FIG. 159.—*Dendrosoma elegans; n*, nucleus. (From Calkins after Kent.)

vegetative life or for reproduction by cell division. Thus amicronucleate races of *Paramecium*, *Didinium*, *Spathidium*, *Oxytricha*, etc., have been maintained for long periods by Woodruff, Dawson, and others, while Maupas, Calkins and others have shown that the micronucleus may disappear in long-continued cultures of hypotrichous forms, although the organisms are still able to divide (p. 72). It is evident that different macronuclei represent different degrees of specialization and that some forms may carry on all processes of asexual activity without a micronucleus and represent transition stages to the opalinids in which there is no nuclear

dimorphism at all and both sexual and asexual processes are possible with only one type of nucleus.

The kinetic elements, including cilia and their derivatives and coördinated systems of intracellular fibrils, represent a neuromotor apparatus quite as complex as that of the higher flagellates. In but few cases are there combinations of other motile organs with cilia. One such case is described by Penard under the name *Myriaphrys paradoxa*, a form with axopodia and cilia (Fig. 160); another is a combination of cilia with a flagellum *Monomastix ciliatus* described by Schewiakoff. The possibility of the derivation of ciliates from flagellates, in some cases through Heliozoa-like forms, is suggested by such types, but origin of this group involves far too much speculation for serious consideration.

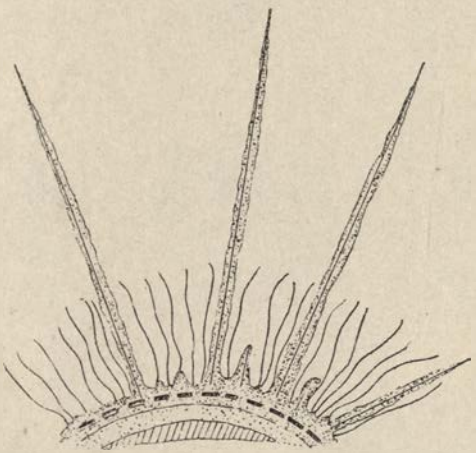

FIG. 160.—*Myriaphrys paradoxa* (?), with cilia and axopodia. (After Penard.)

Cilia, by fusion, form locomotor organs of complex nature (see Chapter III). Undulating membranes, membranelles and cirri, are present in three of the four Orders of ciliates, while undulating membranes are represented in all. A fourth type of combination, membranulæ, combines several of the features of flagella. Thus the powerful motile organs of *Didinium* are composed of a few flagella-like, long cilia, while rhizoplasts run from their basal bodies to the vicinity of the nucleus (Fig. 161, 8).

Undulating membranes are limited regionally, to the gullet, margin of the mouth or to a circumscribed area called the peristome. Membranelles are grouped usually, in a curved row the "adoral zone" around the margin of the peristome, but a dorsal ring of membranelles is present in some parasitic forms (*e. g.*, *Diplodinium*, Fig. 2, p. 20). Cirri are combinations of cilia of usually, the ventral

surface but they may encroach on the dorsal surface (*e. g.*, *Uronychia*); they form groups as a rule, named according to their position, frontal, ventral, anal, and caudal cirri, the number and arrangement forming a basis for diagnosis of genera and species.

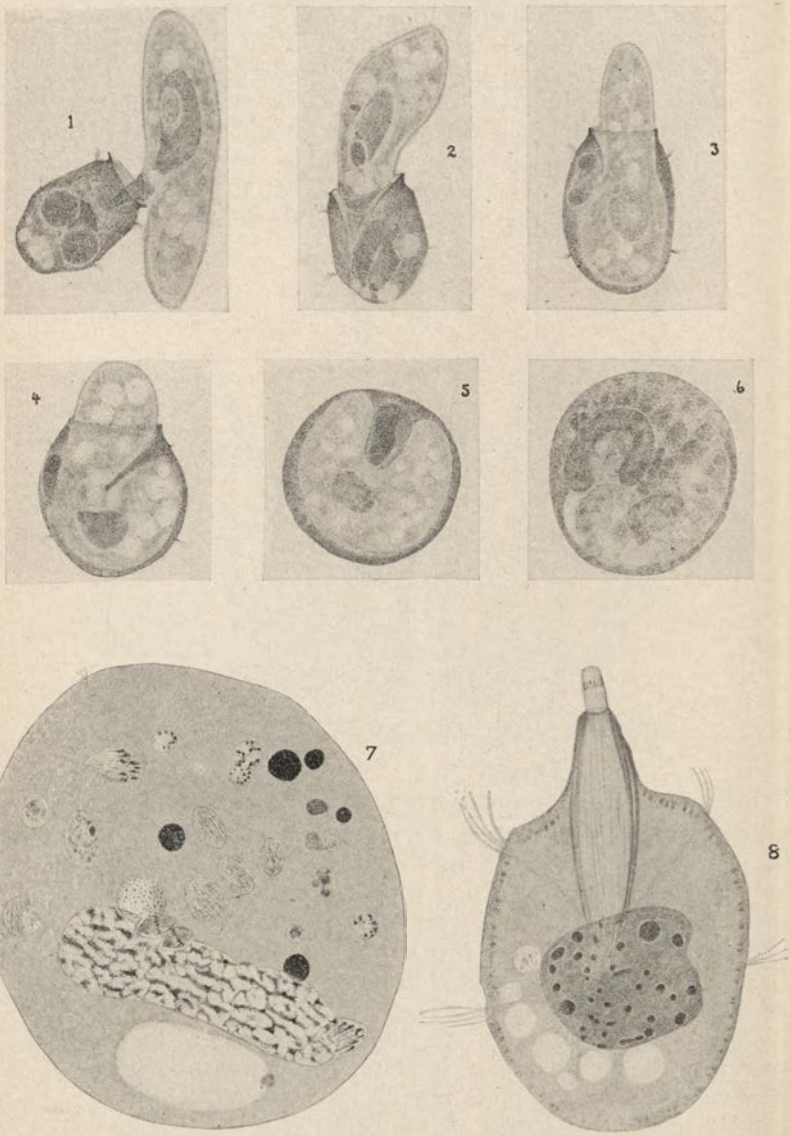

FIG. 161.—*Didinium nasutum* capturing and swallowing *Paramecium caudatum* (1 to 6); 8, section of *Didinium* prior to encystment showing protruded seizing organ with zone of trichocysts and rhizoplasts from the membranulæ to the nucleus. (After Calkins.)

The activities of the motile organs are coördinated through a system of longitudinal and transverse fibrils connecting the basal fibrillæ coming from the cilia or groups of cilia (p. 108). A coördinating center, termed the motorium, has been demonstrated in

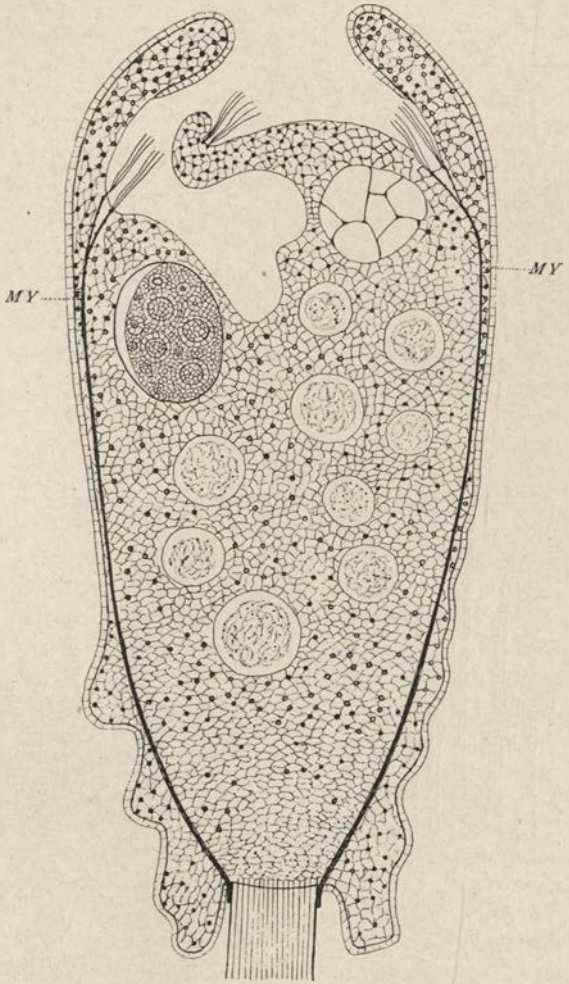

FIG. 162.—*Epistylis plicatilis*. Longitudinal section of individual; *MY*., Myonemes from membranelles to base of cell. (After Schröder.)

some forms (*Diplodinium*, Sharp (1914); *Euplotes* Yocom, 1918; *Balantidium* MacDonald (1922).

Myonemes also are widely distributed in the group. In *Stentor* they lie in superficial canals within the cortex and in some cases appear to be conducting as well as contractile elements. In *Epis-*

tylis, Schröder has described myonemes running longitudinally from the stalk to the peristome where they terminate in the basal plates of the membranelles (Fig. 162); distally they combine to form the contractile strand of the stalk.

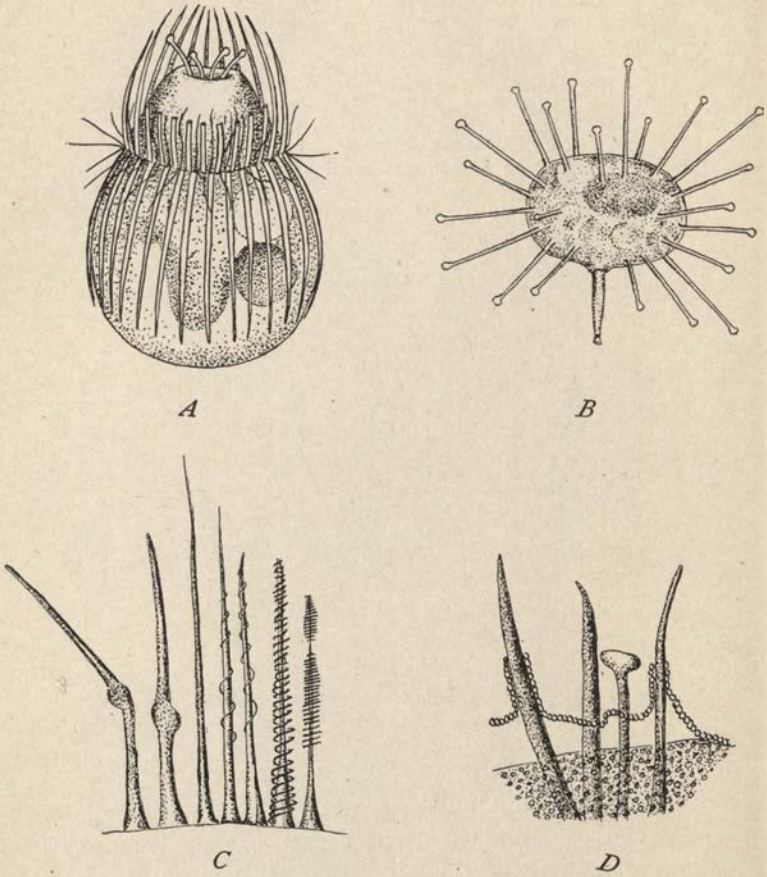

FIG. 163.—Tentacles of Infusoria. *A*, *Mesodinium pulex*, with four oral tentacles for adhering; *B*, *Podophrya fixa; C*, *D*, tentacles of Ephelotidæ. (*A*, *C*, *D*, from Calkins; *B*, Original.)

A well-defined mouth is present in almost all ciliates (absent in an entire group, only in Opalinidæ. In gymnostomata it is closed save at times of food ingestion; in all other groups it is permanently open. In these latter cases the form of the mouth varies from circular to elliptical, crescentic or triangular openings and in the majority of cases leads into a ciliated gullet. Such constant feeders are limited to a bacterial diet and other minute food substances while the gymnostomes, by reason of the distensibility of the oral

region are able to take in living organisms even larger than themselves (see p. 178 and Fig. 161).

In Suctoria, food-taking is of an entirely different type. Mouths are absent but food may be taken in through any one of the many suctorial tentacles. The body wall of a captive organism is cytolyzed at the point where the tentacle is in contact and the endoplasm of the prey either passes in a stream through the lumen of the tentacle, or the endoplasm of the captor enters the body of the victim and digests its endoplasm *in situ* (Maupas, 1883). Tentacles for adhesion are also present in *Mesodinium* (Fig. 163).

While the vast majority of Infusoria are holozoic in food-getting, parasitic types may be holozoic, or saprozoic (*Opalina*). Proteins are digested by all and carbohydrates in some (*Balantidium*, Glaessner, see p. 186).

Parasitism in Infusoria, as in other great groups of Protozoa, is widely spread and some of the adaptations to this end merit special consideration. The majority are apparently harmless commensals of digestive tract and body cavity; some, however, are more serious, *Balantidium coli* for example, causing acute enteritis in man and other mammals. Ectoparasitic forms may also be a source of trouble. *Amphileptus branchiarum* gets under the gill mantles of tadpoles and ingests groups of epithelial cells (Wenrich); others form peculiar arms by which they are anchored to gill bars (*Ellobiophrya donacis*, Chatton). In the main related forms are not strictly parasitic but are attached in gill chambers where a constant supply of food is assured. Special attaching organs, arising from specially modified cilia, are characteristic of holotrichous and of some peritrichous forms. These are best developed in *Trichodina* (common on *Hydra*) where a special attaching organ termed the scopula is characteristic, while the two arms of *Ellobiophrya* mentioned above, are interpreted by Chatton as representing a split scopula. Amongst the Holotrichida, ectoparasitism is characteristic of the group which Chatton calls the Thigmotricha (1923). Here a portion of the posterior ciliated region termed the "thigmotactic area," becomes modified as an attaching organ. It is an adhesive knob in *Ancistrum*, and a protrusible tentacle in *Hypocomides* and *Hypocoma* which Chatton, perhaps correctly, removes from the Suctoria to the Holotrichida. It is rudimentary in *Plagiospira* and not at all evident in *Boveria*. Two types of feeding adaptations are evident in these forms. In one series the peristome and adoral zone become greatly enlarged forming a helicoid spiral in *Boveria*, *Plagiospira*, *Hemispira* and *Ancistrum*, capable of drawing in food particles from a distance. In another series the oral apparatus becomes rudimentary or lost altogether, food substances being absorbed by osmosis through the general body wall or by tentacles only as in *Hypocoma* and *Hypocomides*.

Lumen-dwelling forms have apparently undergone less degeneration than have ectoparasitic types. In the Opalinidæ such degeneration has been the most extreme. Here mouth and other oral structures are entirely wanting and nutrition is osmotic. In the majority of cases however, the peristome and mouth are retained while the cortex is often highly sculptured and fantastic as in the Ophryoscolecidæ.

The aberrant Opalinidæ are parasitic in Amphibia. Not only are they astomatous but in certain characters they differ widely from other ciliates so that they have been variously placed in classification. Hartog (1906) for example placed them with the Hypermastigida of the flagellates. Metcalf (1918, 1923) includes them as Prociliata and sharply marked off from the remaining

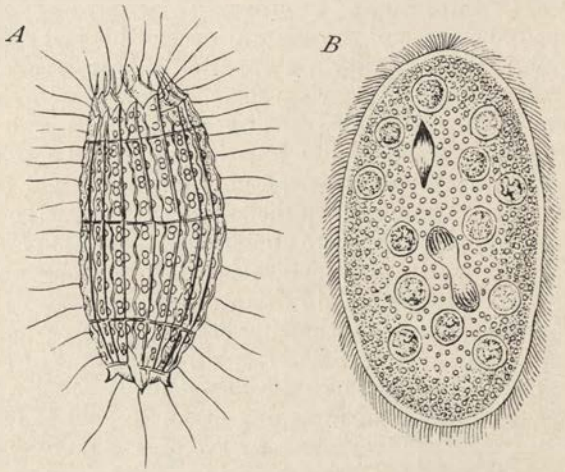

FIG. 164.—*A*, *Coleps hirtus; B*, *Opalina* (?) *ranarum*. (After Bütschli.)

ciliates. In view of the adaptive changes brought about by a parasitic mode of life, it seems more probable that they are degenerate rather than primitive types. There are invariably two or more nuclei but the nuclei are identical with no indication of dimorphism (Fig. 164, *B*). In the nuclei, however, there are two kinds of chromatin according to Léger and Duboscq (1904) and Metcalf (1909 and 1923). The latter distinguishes these types as "macrochromatin" and "microchromatin" the former in mitosis giving rise to band-form "macrochromosomes," the latter to "microchromosomes" in apparently even numbers (from two to ten). The "macrochromatin" is regarded as functional in vegetative life and, like the macronucleus of other ciliates, gives rise to chromidia (Neresheimer) or otherwise fragments preparatory to

absorption in the cell. The "microchromatin" on the other hand is functional during sexual phases. From these considerations it would appear that the dimorphic nuclear conditions of ciliates generally is here represented by each nucleus, a parallel being the condition of nuclei in *Blepharisma undulans* (Calkins, 1912) where the macronuclei with micronuclei enclosed, arise by diffusion of chromatin from products of the amphinucleus (p. 69).

In their sexual phenomena also, the Opalinidæ differ from the majority of other ciliates. Individuals begin to divide rapidly with decreasing size until minute forms result with one, two or more nuclei according to species (Nerescheimer, Metcalf). These encyst, the cysts passing out with the feces. Tadpoles ingest the cysts which open in the rectum giving rise to the same type that had previously encysted. These now multiply, ultimately forming macrogametes and microgametes which fuse on contact. The zygote has one nucleus at first which later gives rise to the binucleated or multinucleated forms, although the exact manner has not been described (Metcalf, 1923).

Reproduction in ciliates generally is typically by binary cross-division and involves a renewal of motile organs, at least this is the case in forms with cirri, and MacDougall (1925) gives evidence to indicate that cilia also are similarly renewed. It thus results that motile organs of both products of cell division are proportionate to the size of the young individuals. Old metaplastids, as pharyngeal baskets, are discarded and are formed anew in both halves. Nuclear changes during division are quite varied, each species having its own peculiarities of macronuclear condensation and reformation (p. 112).

Unequal division or budding while uncommon amongst ciliates is the chief method of reproduction among the Suctoria but also occurs in some Vorticellidæ (*Spirochona*, etc.). In Suctoria, budding is either external or internal, in the latter case the budding area is invaginated, the margins close over, and a brood chamber is formed from which the embryos escape when formed.

Multiple division or sporulation is also uncommon in the Ciliata but occurs in some of the more generalized, and in some parasitic types. When it occurs it is usually under the protection of a temporary cyst (*Colpoda, Ichthyophthirius*).

Sexual processes are practically universal in the group and the main features of the process are similar throughout. In most cases fusion is temporary and pronuclei are exchanged after which the conjugants separate. In some cases, Vorticellidæ, fusion is permanent and sexual dimorphism is the rule, in other cases such dimorphism is expressed by the pronuclei, but in most cases there is no sex differentiation whatsoever (see Chapter XI). In *Trachelocerca phœnicopterus, Ichthyophthirius multifilius*, and in Opalinidæ,

the fertilization phenomena do not follow the usual routine of other ciliates, microgametes being formed and fusion being permanent.

Conjugation always results in physical reorganization of the protoplasm the old macronucleus is broken up and the fragments are absorbed in the cytoplasm while a new macronucleus and new micronuclei are differentiated from products of the first or second division of the amphinucleus after fertilization (see Chapter XI). A similar reorganization takes place at regular intervals of thirty days (*P. aurelia*) or sixty days (*P. caudatum*) according to Woodruff and Erdmann (1914) who termed the phenomena accompanying this method of reorganization "endomixis (p. 540). In other types of ciliates similar asexual processes of reorganization take place under the protection of a cyst (for significance of reorganization see Chapter XII).

The classification adopted here has been generally accepted, and with few changes, since the great work of Bütschli in 1888. We divide the sub-phylum into two classes of unequal size, *viz.*, Ciliata and Suctoria.

Class I. **CILIATA**, Bütschli.

The common features of the Ciliata have been outlined in the discussion above. The distribution and differentiations of the motile organs form the basis for division of the Class into five Orders: Holotrichida; Heterotrichida; Oligotrichida; Hypotrichida and Peritrichida. Motile organs are alike and but slightly differentiated in the Holotrichida where adoral zones are absent. A general covering of cilia, and with an adoral zone of membranelles in addition is the main characteristic of the Heterotrichida; in Oligotrichida body cilia are absent or greatly reduced but an adoral zone is present which as in Heterotrichida and in Hypotrichida turns to the left from the mouth. In Hypotrichida the motile organs are on the ventral surface and are highly differentiated. In Peritrichida an adoral zone which here turns to the right from the mouth alone is present in the ordinary vegetative condition. Further diagnotic features will appear in connection with the different orders.

Order I. **HOLOTRICHIDA**, Stein.

Ciliates with generally uniform body cilia and without a specialized zone of membranelles. Mouth normally present (absent only in Astomina) and with (Trichostomina) or without (Gymnostomina) oral membranes. The mouth may be terminal, subterminal or at any point on the ventral surface or right side. Proboscis-bearing forms are common; spines, bristles and caudal filaments not uncommon; and pharyngeal baskets are highly developed in one family (Chlamydodontidæ). Nutrition is holozoic or parasitic (Astomina); reproduction, normally, is by transverse division. Encystment is

widespread. They are abundant in fresh-water pools, in brackish water, and more rarely, in salt water. Three sub-orders are recognized according to the nature of the mouth or its absence (1) Astomina, (2) Gymnostomina, and (3) Trichostomina.

Sub-order 1. **Astomina.**

Parasitic forms. See Key for genera.

Sub-order 2. **Gymnostomina,** Bütschli.

Holotrichs without undulating membranes in the oral region. The mouth is closed except during ingestion of solid food. In several genera longer cilia are present in the form of an oral circlet. The body is not always covered by cilia, which may be present only on one-half the body, limited to rather widely separated spiral rows, or to one side only. This group includes the most generalized forms of the Infusoria with what may be regarded as the original, terminal position of the mouth. The migrations of the mouth, first suggested by Bütschli with the correlated shifting of the longitudinal rows of cilia affords an interesting study in ciliate morphology. The mouth varies from a small terminal orifice in *Holophrya* (Fig. 166), *Prorodon* (Fig. 165, *C*, *D*, *F*, *G*) or *Didinium*, to a slit occupying the entire anterior end in *Enchelys* (Fig. 166, *C*). This slit is diagonally placed in *Spathidium* (Fig. 90, p. 181), drawn down on the side in *Loxophyllum* (Fig. 167), extended in a long slit down the ventral surface of *Amphileptus* and *Lionotus* (Fig. 167, *A*, *B*) and reduced to a small opening at the base of what may have been an elongated slit, in *Dileptus*. In the Chlamydodontidæ and some Chiliferidæ it has shifted over to the right side while the left side is naked. Some forms are covered with a tightly-fitting cuirass made of sculptured plates, the cuirass dividing with division of the body in *Coleps*, *Tiarina*, etc. (Fig. 164).

Pseudopodia of the axopod type are present in addition to cilia in *Dactylochlamys* and *Myriaphrys*, and plasticity and elasticity are strikingly characteristic of some types, the mouth region of *Lacrymaria olor* for example being capable of stretching out, snake-like, a distance equal to three or four times the length of the body Fig. 76, p. 148).

The Sub-order is divided in four families: (1) Enchelinidæ, (2) Trachelinidæ, (3) Chlamydodontidæ and (4) Nicollellidæ.

Family 1. **Enchelinidæ**, Ehrenberg, Stein.—Generalized forms with most of the striking peculiarities of the Order; body spherical or ellipsoidal and with little distortion of the regular lines of cilia. Nutrition holozoic with deglutition of solid living organisms. Reproduction typical of the Class. Common forms:

1. *Holophrya*, without definite or distinct trichites about mouth (Fig. 166).
2. *Prorodon*, with trichites, or basket, surrounding mouth (Fig. 165).

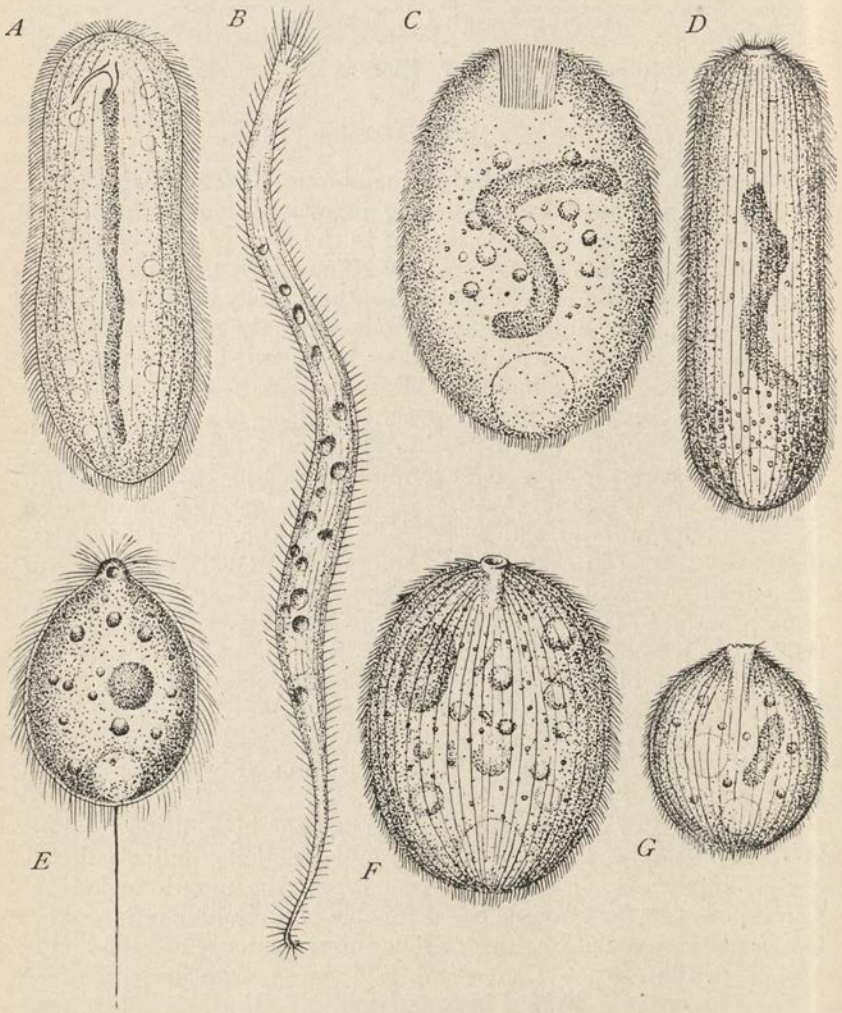

FIG. 165.—Types of Ciliata. *A*, *Hoplitophrya lumbrici*; *B*, *Trachelocerca phœnicopteris*; *C*, *Prorodon niveus*; *D*, *Prorodon farebus*; *E*, *Urotricha farcata*; *F*, *Prorodon teres*; *G*, *Prorodon armatus*. (*A*, *C*, *D*, *E*, *F*, *G*, after Bütschli; *B*, after Calkins.)

3. *Actinobolus*, with long tentacles while at rest (Fig. 81, p. 154).
4. *Coleps*, with cuirass of plates, posterior truncated . th spines (Fig. 164).

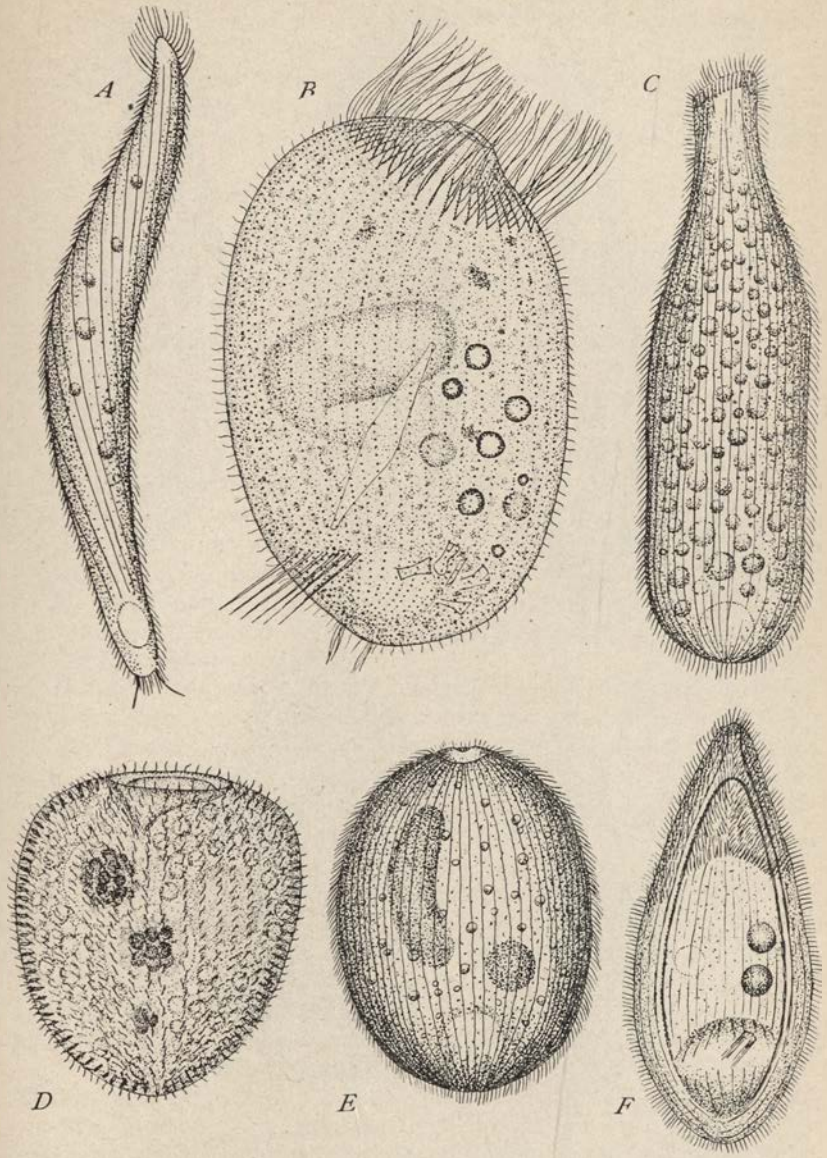

FIG. 166.—Types of Ciliata. *A*, *Chœnia teres;* after Calkins; *B*, *Cyclotrichium ovatum*, after Fauré-Fremiet; *C*, *Enchelys pupa*, after Bütschli; *D*, *Holophrya gargamellæ*, after Fauré-Fremiet; *E*, *Holophrya discolor*, and *F*, *Opisthodon mnemiensis*. (After Bütschli.)

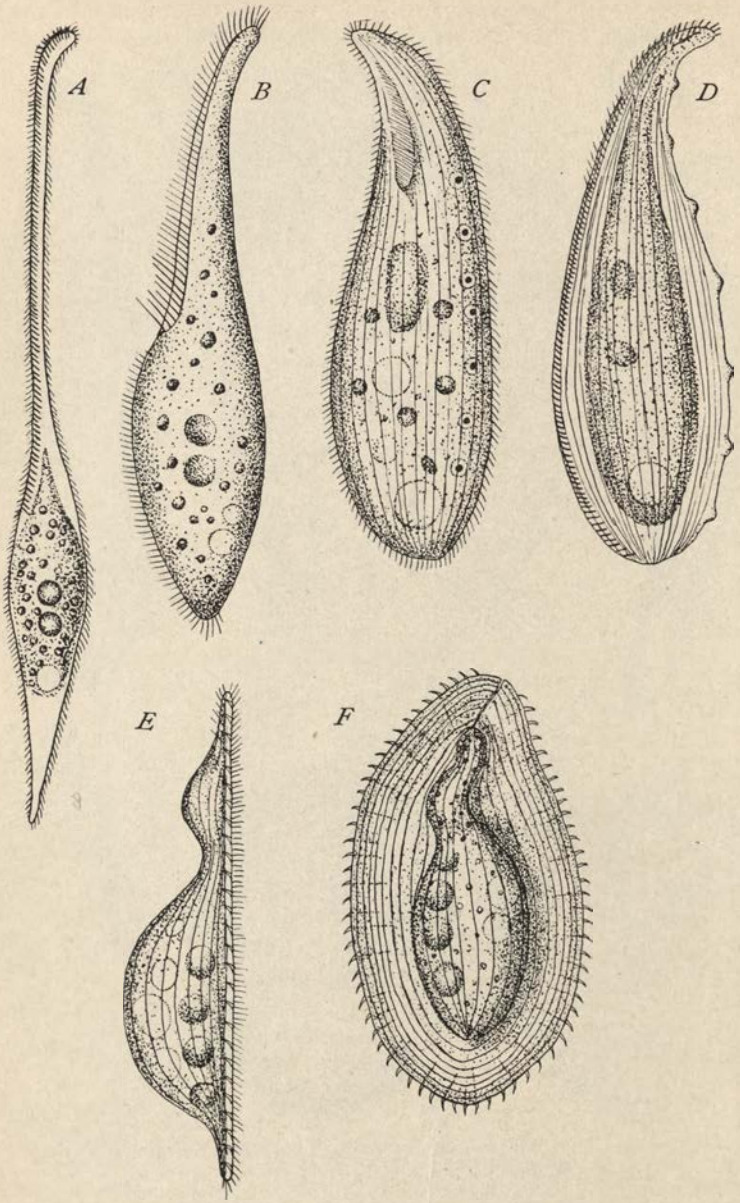

FIG. 167.—Types of Ciliata. *A*, *Lionotus wrzesniowskyi; B*, *Lionotus fasciola; C*, *Loxodes rostrum; D*, *Loxophyllum meleagris; E*, and *F*, *Loxophyllum setigera.* (*A*, and *C*, after Bütschli, the others after Calkins.)

5. *Tiarina,* with cuirass of plates posterior end pointed.
6. *Plagiopogon,* longitudinal plates; without spines.
7. *Chœnia,* highly plastic; mouth circular; no proboscis (Fig. 166, p. 379).
8. *Lacrymaria,* with neck-like constriction bearing circlet of cilia (Fig. 76, p. 148).
9. *Trachelophyllum,* very elongate; knob at end of neck-like region.
10. *Trachelocerca;* flexible, with circlet of cilia, no constriction.
11. *Metacystis,* similar to 10 but not flexible.
12. *Lagynus,* anterior sharply truncate; body ellipsoidal.
13. *Enchelys,* anterior sharply truncate; body flask-shape (Fig. 166, *C*).
14. *Spathidium,* mouth an oblique slit occupying entire anterior end (Fig. 90, p. 181).
15. *Monodinium,* cilia reduced to one girdle about proboscis (Fig. 75, p. 146).
16. *Didinium,* cilia reduced to two girdles (Fig. 88, p. 179).
17. *Balanitozoön,* cilia absent from posterior third of body.
18. *Mesodinium,* one central girdle of cilia (Fig. 163, p. 372).
19. *Pelamorpha,* test-dwelling; body with posterior filaments.
20. *Urotricha,* uniformly ciliated with caudal bristle (Fig. 165, *E*).
21. *Myriaphrys,* with cilia and axopodial pseudopodia.
22. *Dactylochlamys,* cilia and lobopodia; sapropelic.

For other Genera see Key.

Family 2. **Trachelinidæ**, Ehrenberg, Stein.—Generalized forms with a uniform coating of cilia and with the anterior end drawn out in the form of a proboscis. The mouth may be a slit on the ventral side of the proboscis or a circular opening on dorsal (*Trachelius*) or ventral surface (*Dileptus*, etc.). Reduction of cilia confined to comparatively few forms (*Lionotus, Loxophyllum*). Figures pages 378, 379, 380.

Common Genera: Loxodes, with an indefinitely indicated proboscis; *Trachelius,* with a short and abrupt proboscis: *Lionotus* and *Amphileptus* with the mouth in the form of an elongated slit extending the entire length of the proboscis; *Dileptus* with circular mouth at the base of the proboscis, and *Loxophyllum* with flattened body and short slit-like mouth, and without definite proboscis.

Family 3. **Chlamydodontidæ**, Stein, Bütschli.—Generally flattened forms with cilia reduced to the right side which, bearing the mouth, is the functional ventral side. The mouth is surrounded by a basket-work of trichites the bases of which extend deeply into the endoplasm.

Common Genera: (For other genera see Key.)

1. *Nassula* (Fig. 13, p. 35), body ellipsoidal or only slightly flattened.

2. *Opisthodon* (Fig. 166, p. 379), flattened; mouth near posterior end of the body.
3. *Chlamydodon*, flattened with hyaline band running around the margin.
4. *Orthodon* (Fig. 83, p. 158), flattened with mouth on right side of anterior angle.
5. *Chilodon* (Fig. 106, p. 225), flattened with mouth near center of right side.
6. *Trichopus*, with a ciliated brush as a caudal appendage.
7. *Dysteria*, with spine-like process and narrow ventral surface.
8. *Phascolodon*, with spine-like process and broad ventral surface.
9. *Ægyria*, with spine; uniform cilia on ventral surface.
10. *Scaphidiodon*, with spine; dorsal ridge or keel prominent; ventral bands.
11. *Trochilia*, with spine; ventral cilia in bands; no dorsal keel.

Family 4. **Nicollellidæ**.—Parasitic forms of African rodents.

Sub-order 3. **Trichostomina.** Bütschli.

In these forms the mouth is permanently open and provided with one or more undulating membranes which run into the gullet or border the mouth. The majority are free-living but some are parasitic. The cilia are rarely reduced but usually cover the body. In the genus *Urocentrum*, however, they are limited to a zone in the anterior half and a larger broader zone in the posterior half. A well-marked peristomial depression is frequent, and a spiral twisting of the body characteristic. The undulating membranes are narrow and inconspicuous for the most part but in some cases form great balloon-like sails used apparently, for the trapping of food (*Pleuronema*, *Lembadion* Fig. 169, p. 385), *Pleurocoptes*, etc.).

Six families are recognized, one, however, consists of the single genus *Urocentrum* (Urocentridæ, Fig. 168, *D*). The others, with their typical genera are:

Family 2. **Chiliferidæ**, Bütschli.—Here the body is uniformly ciliated and has no peristomial furrow. The mouth is in the anterior half of the body which may be flattened, ovoidal, ellipsoidal, cigar-shape or kidney-shape. The genera are:

1. *Leucophrys*, mouth the entire anterior end, body flattened, flask-shape.
2. *Glaucoma* (Fig. 168, *E*), ellipsoidal; mouth triangular; two oral membranes.
3. *Ophryoglena* (Fig. 168, *A*), ellipsoidal; mouth circular or crescentic, subterminal.
4. *Dallasia* (Fig. 168, *B*), cigar-shape; mouth subterminal.

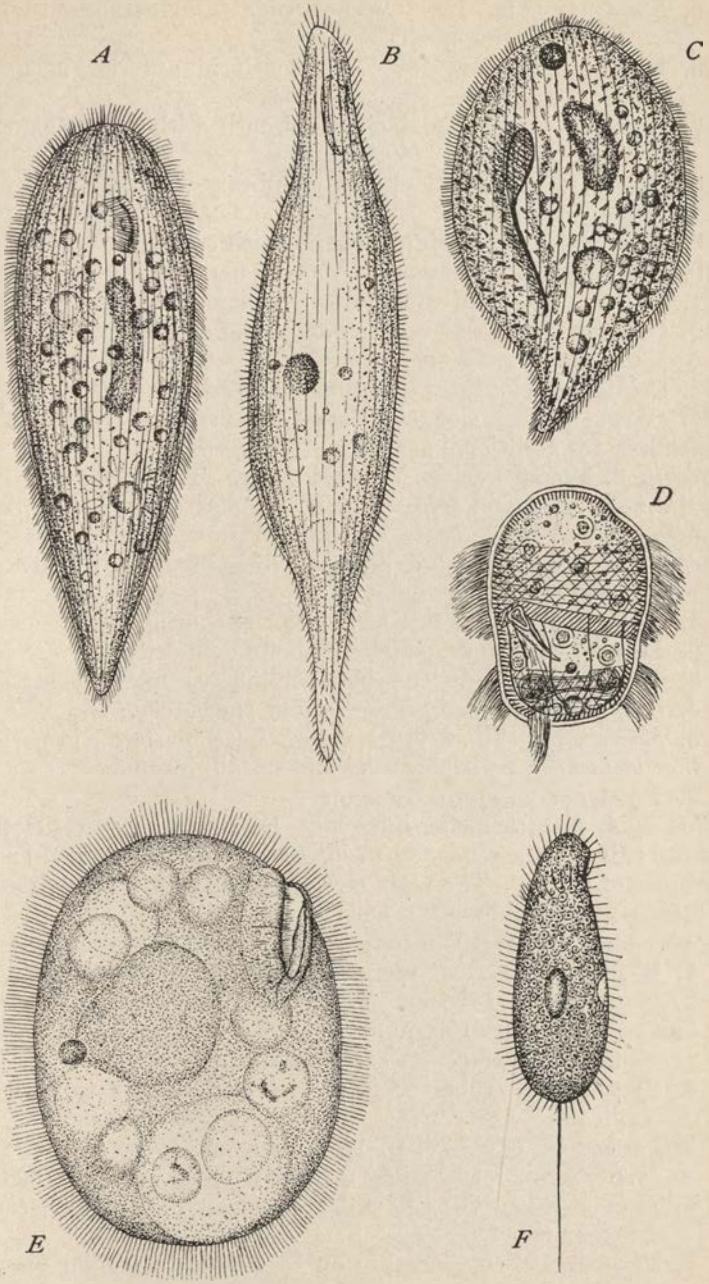

FIG. 168.—Types of Ciliata. *A*, *Ophryoglena flava; B*, *Dallasia frontina; C*, *Frontonia acuminata; D*, *Urocentrum turbo; E*, *Glaucoma* sp.; *F*, *Loxocephalus granulosus.* (*A*, *C*, *D*, and *F*, after Bütschli; *B*, from Conn after Stokes; *E*, original.)

5. *Frontonia* (Fig. 168, *C*), ellipsoidal; elongate mouth with furrow running posteriorly.
6. *Loxocephalus* (Fig. 168, *F*), mouth in anterior half; with caudal spine.
7. *Uronema*, with caudal cirrus; mouth with marginal membrane.
8. *Placus*, with caudal cirrus; membrane cross-striped, plaid-like.
9. *Colpodopsis*, body kidney-shape, with caudal bristle.
10. *Plagiopyla*, peristomial furrow very narrow and transverse.
11. *Tillina*, spheroidal with posterior, pigmented, dorsal lobe.
12. *Colpoda*, body distinctly kidney-shape; oral region deeply insunk.
13. *Colpidium*, elongate; dorsal side arched, oral region shallow.
14. *Chasmatastoma*, elongate; dorsal side flat, oral region shallow.

(See Key for additional genera.)

Family 3. **Microthoracidæ**, Wrzesniowsky.—These forms are small for the most part and frequently of peculiar shape—lens, sickle, triangle, etc. The body is compressed except in the cases of *Boveria* and *Plagiospira* and accessory bristles are frequent. The main genera are:

1. *Ancistrum*, with a tuft of anterior anchoring cilia.
2. *Boveria*, with short peristome; body cylindrical.
3. *Cinetochilum*, body lens-shape, obliquely truncate posteriorly.
4. *Drepanomonas*, body crescentic or sickle-form.
5. *Microthorax* (Fig. 170, *A*), mouth small, posterior in position.
6. *Plagiospira*, peristome long and spirally wound.
7. *Ptychostomum*, form triangular.

Family 4. **Pleuronemidæ**, Bütschli.—Forms with general clothing of long cilia with a tendency to longer and more powerful cilia in the anterior region. The main characteristic is the presence of one or more powerful undulating membranes which may be folded into the peristome on the left anterior side. The typical genera are:

1. *Blepharostoma*, with one pseudo-membrane (close set cilia).
2. *Calyptotricha*, tube or jelly-dwelling; no long anterior cilia.
3. *Cyclidium* (Fig. 169, p. 385), narrow peristome; one balloon-like membrane; with caudal filament.
4. *Cyrtolophosis*, tube- or jelly-dwelling; anterior tuft of long cilia.
5. *Lembadion* (Fig. 77, p. 149), peristome very broad; three undulating membranes; with or without caudal filament.
6. *Lembus* (Fig. 170, *C*), two pseudomembranes; with caudal filament.
7. *Pleurocoptes*, ectoparasitic on hydroids; no caudal filament and without balloon-like membrane.
8. *Pleuronema* (Fig. 169, *C*), no caudal filament; with one balloon-like membrane.

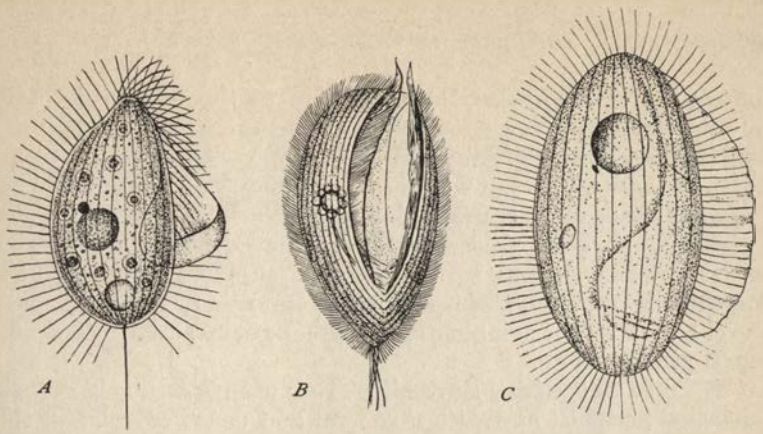

FIG. 169.—Types of ciliates. *A*, *Cyclidium glaucoma; B*, *Lembadion bullinum; C*, *Pleuronema chrysalis*. (*A*, *C*, after Calkins; *B*, after Bütschli.)

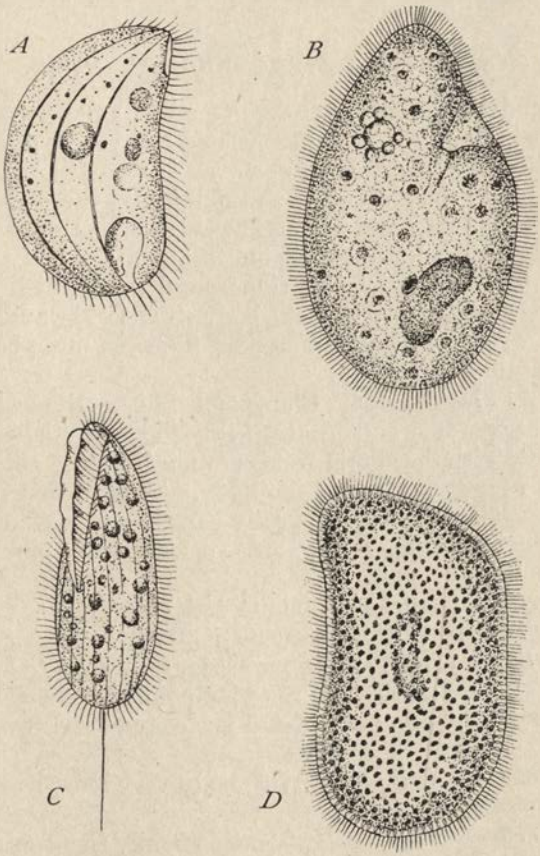

FIG. 170.—Types of Ciliata. *A*, *Microthorax sulcatus; B*, *Paramecium putrinum C*, *Lembus pusillus; D*, *Paramecium bursaria*. (*A*, *B*, *D*, after Bütschli; *C*, after Calkins.)

Family 5. **Paramecidæ**, Bütschli.—Up to the present including only the genus *Paramecium* of which the common forms *P. caudatum* and *P. aurelia* are elongate and cigar-shape with a spirally-wound peristome and a gullet in which a single undulating membrane lies. *P. putrinum* is shorter, more plump, and lacks the spirally wound peristome. *P. bursaria* is flattened somewhat kidney-shape, has one micronucleus, and is usually well supplied with symbiotic Zoöchlorella. *P. calkinsi* is similar to *bursaria* but has two micronuclei and lacks the symbionts. Star-shape contractile vacuoles and canals present in all.

Family 6. **Isotrichidæ**, Bütschli—These are parasitic ciliates with thickened periplast and with a general and dense covering of cilia. They live, probably as commensals, in the fore-stomach of ruminants.

(See Schuberg, 1888, or Eberlein, 1895 for genera.)

Order II. **HETEROTRICHIDA**, Stein.

In this and the following Orders of ciliates the peristome bears on its left margin, a row of differentiated aggregates of cilia termed membranelles the row being known as the adoral zone. In this Order the adoral zone is wound to the left and the body is covered with a coating of fine cilia which, however, as in Holotrichida, may be variously reduced. The varying forms of the body are due, in the main, to modifications of the frontal field which may be at right angles to the long axis of the body (Stentoridæ and Bursaridæ) or parallel with it. These differences form the main basis of division into families as follows:

Family 1. **Plagiotomidæ**, Clap. and Lach., Bütschli.—In this family the peristome and frontal field is characteristically narrow and elongate with the adoral zone running from the anterior end to the mouth near the center of the body. Typical Genera:

1. *Conchophthirius*, body laterally compressed; anterior rounded.
2. *Blepharisma* (Fig. 174, p. 391), color usually pink; peristome straight; pointed at end.
3. *Helicostoma*, peristome oblique with spiral gullet.
4. *Metopus* (Fig. 171, *B*), body distinctly twisted; not contractile.
5. *Nyctotherus* (Fig. 171, *C*), parasitic; form oval to bean-shape.
6. *Plagiotoma*, parasitic(?); anterior pointed, posterior truncate.
7. *Porpostoma*, body not twisted nor compressed; two crescentic oval lips.
8. *Spirostomum* (Fig. 30, p. 70), body very long, band-form and highly contractile.

Family 2. **Stentoridæ**, Stein.—In this family the frontal field is at right angles to the long axis of the body, or in *Climacostomum* at a marked angle with such axis. In some genera the frontal field is

drawn out into arms or processes. The macronucleus is almost always beaded. Typical genera are:

1. *Climacostomum* (Fig. 56, p. 107), with flattened body; frontal field at an angle.

2. *Fabrea*, body pear-shape, widest at the base; attached.

3. *Folliculina* (Fig. 84, p. 160), frontal field drawn out into arms; attached; tube dwelling.

4. *Stentor* (Fig. 74, p. 145), body trumpet-shape when expanded.

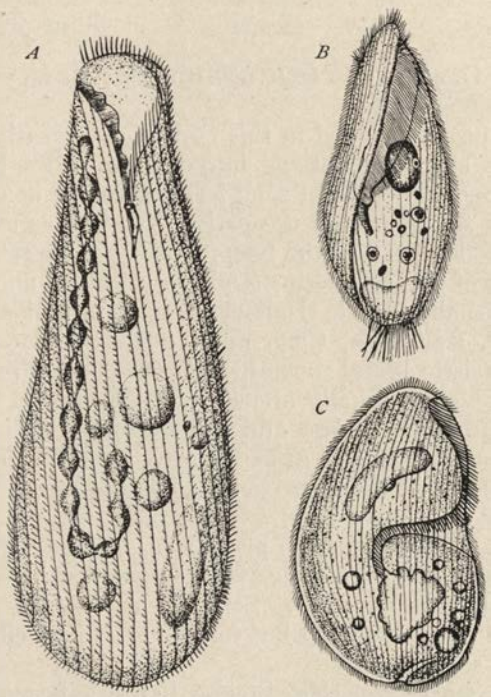

FIG. 171.—Types of Ciliata. *A*, *Condylostoma patens; B*, *Metopus sigmoides; C*, *Nyctotherus cordiformis.* (*A*, after Calkins; *B*, *C*, after Bütschli.)

Family 3. **Gyrocorycidæ**, Stein.—A family created for the reception of the single genus *Cœnomorpha* of Perty (= *Gyrocoris*, Stein), with characteristic bell-shape body and manubrium-like tail or process; a second genus *Trochella* of Penard has been added.

Family 4. **Bursaridæ**, Stein.—In this family the peristome is wide and usually deeply insunk. The representatives are either free-living or parasitic; of the latter *Balantidiopsis* is laterally compressed and the peristome inconspicuous, while in *Balantidium* the body is oval or ellipsoidal, and the peristome only slightly insunk. Of the free-living forms *Bursaria* has a peristome so deeply insunk

as to give to the body the effect of a bag or sac. In *Condylostoma* the peristome is triangular and the peristome is only slightly insunk (Figs 84, p. 160; 171, p. 387).

Family 5. **Ctenostomidæ**, Lauterborn.—The genera of this family —*Discomorpha, Saprodinium, Pelodinium* and *Epalxis*—are peculiarly modified forms typical of the sapropelic fauna. The body is laterally compressed and asymmetrical and the cilia are reduced while spines and a peculiar comb-like structure above the mouth distinguish them from all other ciliates.

Order III. **OLIGOTRICHIDA**, Bütschli.

The organisms included in this Order have greatly reduced cilia or none at all, combinations into membranelles being the sole motile organs. The adoral zone forms a nearly or quite complete ring around the margin of the peristome which is usually at right angles to the long axis of the body. Of the three families included one consists of parasitic forms (Ophryoscolecidæ) and two are free-living and usually pelagic (Halteriidæ and Tintinnidæ).

Family 1. **Halteriidæ**, Clap. and Lach.—Small organisms with the characteristic adoral zone of the Order and with no cilia on the peristome which is usually arched. The pellicle is hardened into a test in the genus *Labœa* and a zone of protective trichites is present in some species of *Strombidium*. Spines, cilia and cirri are generally absent in these two genera but are present in the genus *Halteria* (for Genera see Key).

Family 2. **Tintinnidæ**, Clap and Lach. emend.—These are small pelagic forms, usually marine, with frequently highly sculptured tests and spinous processes. Some forms have a few scattered rows of cilia down the body but for the most part cilia are absent. (For genera see Key.)

Family 3. **Ophryoscolecidæ**, Stein.—Parasitic forms of frequently fantastic shape with a thick periplast and a retractile peristome; cilia are generally absent, the adoral zone is a complete circle and in some forms there is an additional ring of membranelles apart from the peristomial apparatus. The posterior end is often drawn out into spines and processes of peculiar shape and arrangement.

The genera *Entodinium* and *Cycloposthium* have but one circle of membranelles, the former with posterior spines, the latter with two bundles of latero-posterior appendages. The genera *Ophryoscolex* and *Diplodinium* have two circlets of membranelles, the second circlet forming an incomplete ring posterior to the adoral zone in *Ophryoscolex*, and a complete ring on the dorsal side in *Diplodinium*. (For genera see Key.)

Order IV. HYPOTRICHIDA, Stein.

The ciliates included in this Order belong to the most highly differentiated forms of the Protozoa. Except for some species belonging to the more generalized types (*e. g.*, *Uroleptus mobilis*) they are flattened dorso-ventrally and bear motile organs only on the ventral surface. Tactile organs may be present on the dorsal side but these are not used for locomotion. They may be easily distinguished from the laterally flattened Holotrichida (Chlamydodontidæ) by the presence of a usually powerful adoral zone of

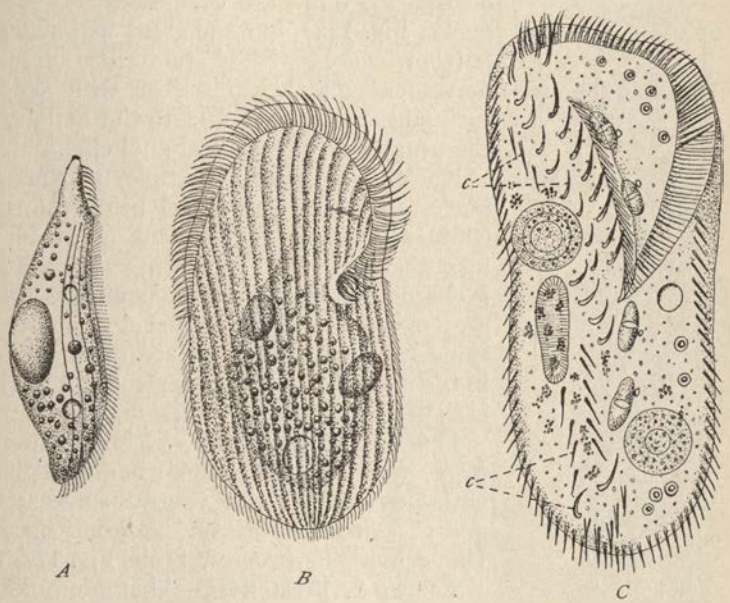

Fig. 172.—*A*, *Stephanopogon colpoda; B*, *Peritromus emmæ; C*, *Onychodromus grandis*. *c*, Cirri. (From Calkins after Bütschli.)

membranelles which is spirally wound to the left. The frontal field is usually triangular and bears in the more complex types, one or more undulating membranes. The genera and species offer an excellent opportunity for the study of comparative anatomy through homology. Thus the most generalized forms, represented by types such as *Peritromus* have no other motile organs than the close-set rows of ventral cilia of uniform size, and the adoral zone of membranelles (Family Peritromidæ) (Fig. 172). In other types localized areas of cilia are represented by cirri, the origin of which is generally attributed to the fusion of adjacent cilia. Such areas give rise to regional groups of cirri known as frontals, ventrals,

laterals, anals and caudals and there is a reduction in the number of uniform ventral cilia corresponding to the increased number and complexity of the cirri. Thus frontals appear as the only differentiated motile cirri in some species of the Urostylidæ, frontals and anals in other species, while the remainder of the ventral surface is clothed with uniform cilia. Cirri are increased and ventral cilia further reduced in the Family Pleurotrichidæ, and ventral cilia are entirely lost in the family Euplotidæ and in Aspidiscidæ.

Fig. 173.—*Stichotricha secunda*, a tube-dwelling hypotrichous ciliate. (Original.)

The hypotrichs are rarely parasitic (*Kerona* on *Hydra*); a few are tube-dwelling (*Stichotricha*, Fig. 173), but the great majority are bottom feeders with characteristic creeping movement on their cirri, or with sudden springing movement due to the activity of the usually more powerful anal cirri.

Nuclei are usually multiple, two macronuclei and two micronuclei being the rule; conjugation and encystment occur in all forms, and, so far as known, reorganization is characteristic of both phenomena. Cysts are frequently ornamented by numerous spines. The six families are distinguished, in the main, by the arrangement and specialization of the ventral motile organs.

Family 1. **Peritromidæ**, Stein.—Flattened forms with coating of uniform and undifferentiated cilia on the ventral surface; the adoral zone follows the anterior margin. One genus—*Peritromus*, Stein Fig. 172, *B*).

Family 2. **Urostylidæ.**—This group differs from the more generalized *Peritromus* in the differentiation of the frontals while anal cirri may or may not be present. Ventral cilia are present in all forms. Some are ectoparasitic (*e. g.*, *Kerona pediculus*, Fig. 79, p. 151, on *Hydra*) but the majority are free-living in stagnant water. Characteristic genera are given below; see Key for full list.

Anal cirri are present only in the genera:

1. *Amphisia* (Fig. 175), with 2 to 3 rows of ventral cilia, 2 marginal rows.

2. *Urostyla*, with 5 to 7 rows of ventral cilia, 2 marginal rows. Three to several frontal cirri, but no anal cirri are present in:

3. *Trichogaster*, with ventral surface covered with fine cilia.

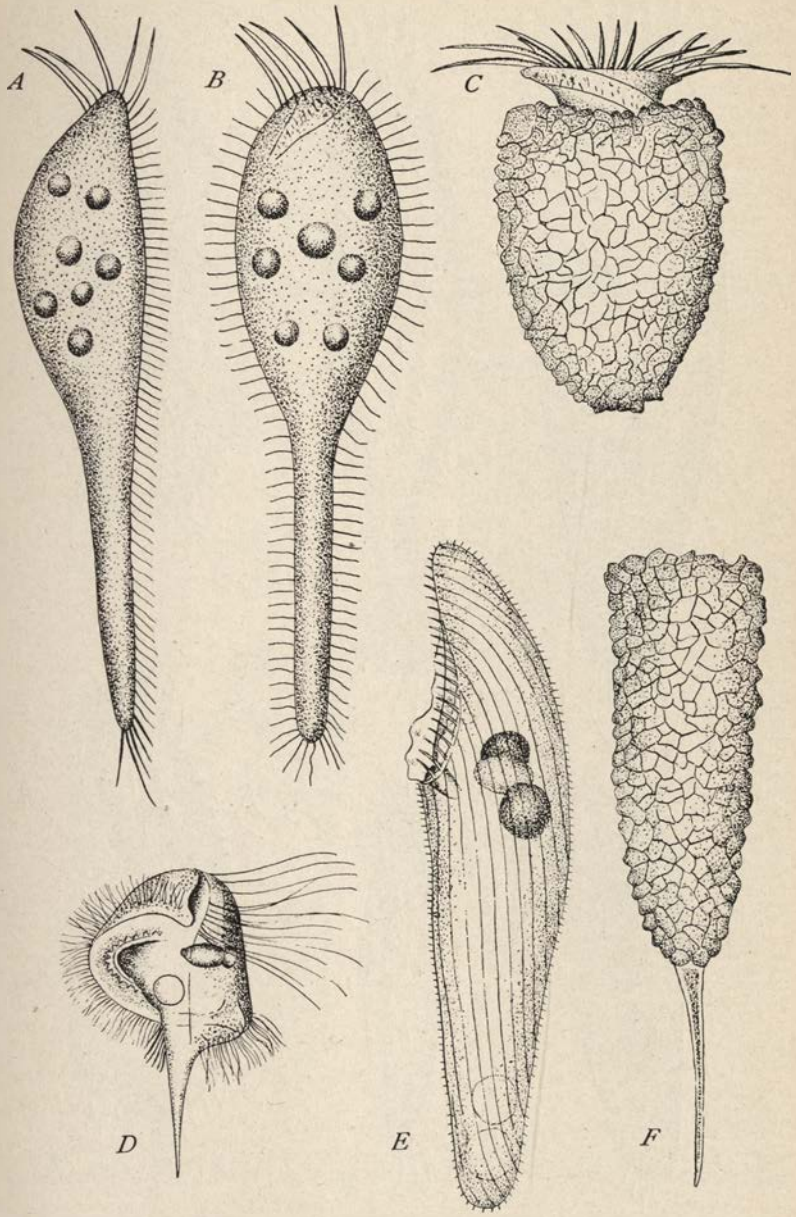

FIG. 174.—Types of Ciliata. *A* and *B*, *Epiclintes radiosa; C, and F*, species of *Tintinnopsis; D*, *Cœnomorpha medusula; E*, *Blepharisma undulans.* (*A*, *B*, *C*, *E*, and *F*, after Calkins; *D*, after Bütschli.)

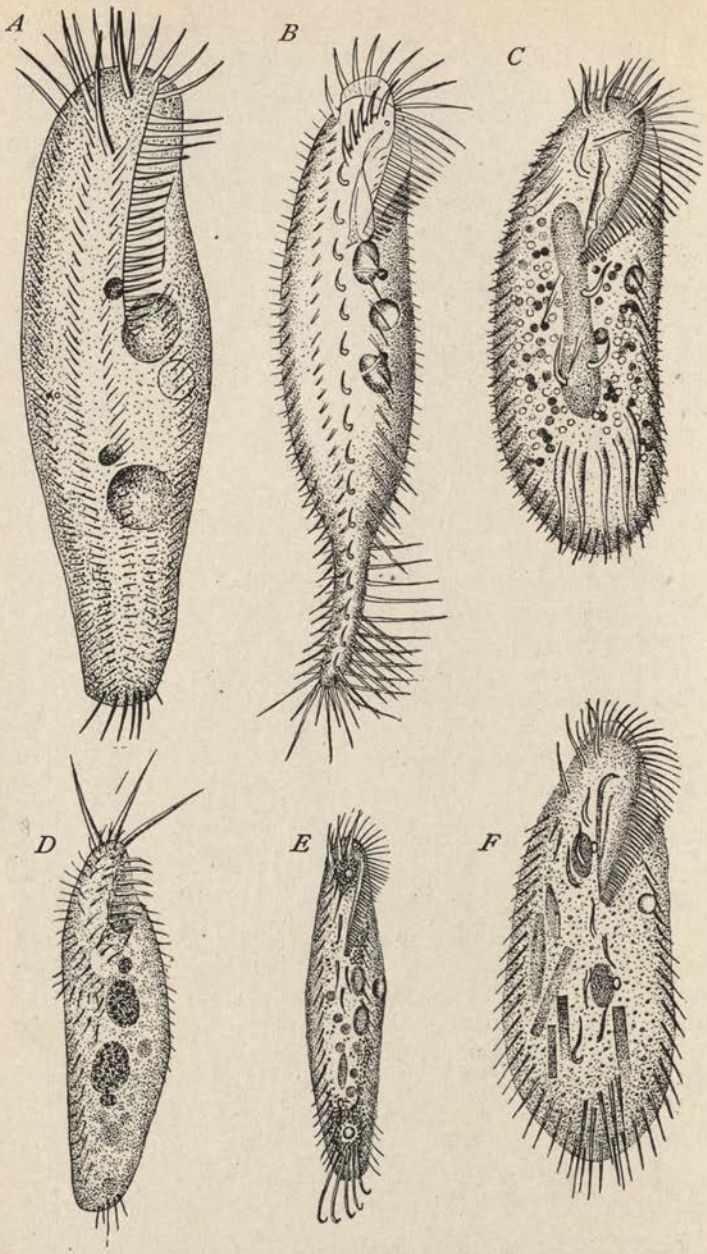

FIG. 175.—Types of Ciliata. *A*, *Amphisia kessleri; B*, *Uroleptus pisces; C*, *Histrio pellionella; D*, *Strongylidium* sp.; *E*, *Oxytricha pellionella; F*, *Oxytricha fallax.* (*A*, after Calkins, *B*, *C*, *D*, *E*, after Bütschli; *F*, after Stein.)

4. *Uroleptus* (Fig. 1, and Fig. 105, p. 222), with 4 or 5 rows of ventral cilia, no caudal bristles.

5. *Strongylidium*, 4 to 5 rows of ventral cilia; with caudal bristles. Differentiated frontal cirri are absent in:

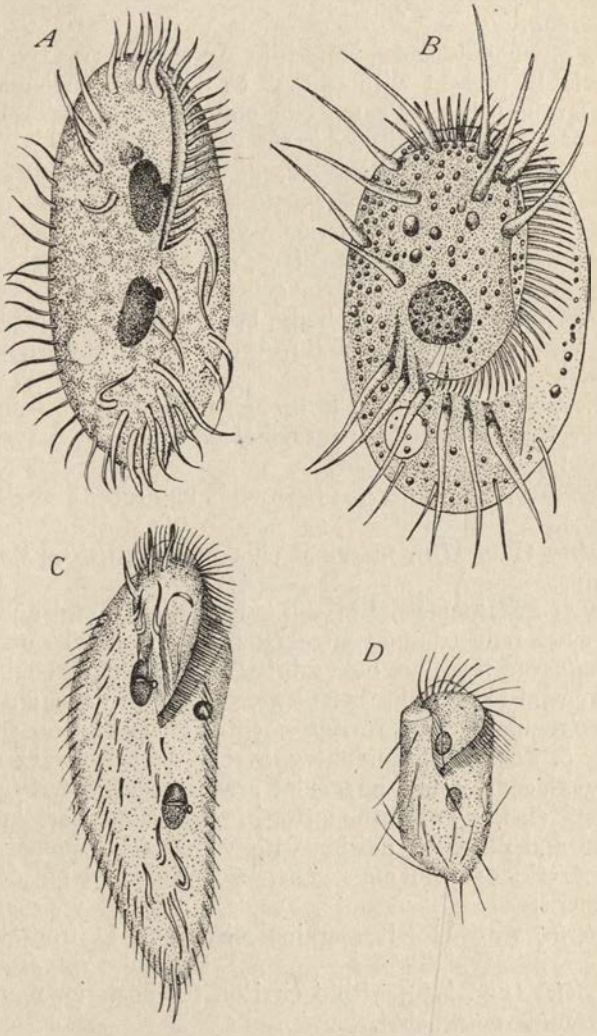

Fig. 176.—Types of Ciliata. *A*, *Gastrostyla steinii; B*, *Euplotes vannus; C*, *Pleurotricha lanceolata; D*, *Psilotricha acuminata.* (*A*, *B*, after Calkins; *C*, *D*, after Stein.)

6. *Epiclintes* (Fig. 174), cilia rows straight; body with neck and tail.

7. *Holosticha*, cilia rows straight; body without neck or tail.

8. *Stichotricha* (Fig. 173), cilia rows spiral; often tube-dwelling; peristome long.

9. *Sparotricha*, cilia rows spiral; peristome half the length of proboscis.

Parasitic; rows of cilia spiral.

10. *Kerona*.

Family 3. **Pleurotrichidæ**, Bütschli.—In these forms the anal cirri are invariably present with from 5 to 8 frontal cirri and 1 or 2 rows of marginal cilia. Ventral cilia may be present in broken rows.

The posterior end is tail-like in the genus:

1. *Urosoma*, in all other genera a distinct tail is absent.

The peristome is very narrow in the genera:

2. *Actinotricha*, with 5 anterior ray-like spikes (membranelles).

3. *Gonostomum*, no anterior spikes; with 3 caudal cirri.

The peristome is wide in the genera:

4. *Gastrostyla;* with flexible body, broken rows of ventral cilia.

5. *Oxytricha* (Fig. 175), with 8 frontals, 5 ventral cirri, and short caudal cirri.

6. *Stylonychia;* marginal cilia broken posteriorly; 3 caudal cirri.

7. *Pleurotricha* (Fig. 176); marginal cilia unbroken posteriorly; anals 3 and 2.

8. *Onychodromus* (Fig. 172); both ends truncate; 5 anals in line; large forms.

9. *Histrio* (Fig. 175), marginal cilia unbroken; 5 anals in line; small forms.

Family 4. **Psilotrichidæ**, Bütschli.—Here the frontal and ventral cirri are much reduced and ventral cilia are entirely absent. In the genus *Balladina* there are heavy bristle-like marginal cirri and one row of ventral cirri, while in the genus *Psilotricha* (Fig. 176, *D*) there is no regularity in the arrangement of cirri which are scattered.

Family 5. **Euplotidæ**, Ehrenberg, Stein.—In this family cilia are entirely replaced by cirri in regular arrangement of frontals, marginals, ventrals and anals, the latter in some cases becoming highly developed and powerful, uniting with ventrals or caudals to form a complex series of springing organs. Giant cirri are not developed in the genera:

1. *Certesia*, with 11 left marginal cirri, and 11 frontal-ventral cirri.

2. *Euplotes* (Fig. 176), with 4 cirri on the posterior margin and 9 to 10 frontal-ventral cirri.

Giant cirri are present in the genera:

3. *Diophrys*, with 3 giant posterior, and 7 to 8 frontal-ventral cirri.

4. *Uronychia* (Fig. 107, p. 225), with 7 to 9 giant posterior cirri.

Family 6. **Aspidiscidæ**, Stein.—In this family the individuals are comparatively small and are characterized by a peculiar sculpturing

on the ventral surface whereby a shoulder-like ledge bounds the cirri-forming area on the right side. In the genus *Onychaspis* (Fig. 80, p. 152), the cirri are brush-like aggregates of loosely associated cilia directed at right angles to the long axis of the body and the peristome begins at the middle of the left margin. In the genus *Aspidisca*, the cirri are directed parallel with the long axis; the anals are numerous (from 5 to 12) and the peristome begins at the anterior end.

Order V. **PERITRICHIDA**, Stein.

The adoral zone of membranelles of a heterotrich or of a hypotrich from the gullet, turns to the left if viewed from the ventral or peristomial side but in the Peritrichidæ, with few exceptions, the adoral zone if viewed from the ventral side, turns to the right. How this peculiar reversal came about is a matter of speculation. Bütschli and Fauré-Fremiet have attempted to explain it on a phylogenetic basis and at the same time to account for the apparent longitudinal division of forms like the Vorticellidæ. The former interpreted the reversal as an adaptation of a flattened hypotrichous form in which the ventral surface serves for attachment while the peripheral region of the adoral zone becomes turned over on the dorsal side. The functional ventral surface would thus be the morphological dorsal surface and the attaching surface the morphological ventral surface (*Trichodina* for example). In the Vorticellidæ the ventral surface becomes drawn out into an attaching stalk and the body becomes elongated in the dorso-ventral plane. Division therefore in a morphological sense, would be transverse rather than longitudinal. Fauré-Fremiet's (1905) explanation is based on forms like *Ancistrum*, *Hemispira* and other holotrichs with an area of attaching cilia (thigmotactic area) and with the tendency of mouth and peristome to turn upward.

As a result of an attached mode of life, colony formation, unique amongst ciliates, is characteristic of the Vorticellidæ. Here, also, under conditions as yet unknown, the individual cells may leave their stalks after developing a girdle of posterior cilia and swim away as solitary individuals. Except for this temporary ciliated girdle, body cilia are rarely present but are characteristic of *Trichodinopsis* and *Hemispira*. Houses or tests are present in all species of Cothurnina, but are generally absent in other groups.

In their sexual phenomena the Peritrichida differ in some important respects from other ciliates. In the majority of cases in which fertilization processes have been worked out, dimorphic gametes are formed and fusion is complete and permanent. Mutual fertilization is thus absent and corresponding changes in the maturation phenomena are introduced (see Chapter XII).

The great majority of Peritrichida are placed in the family Vorti-

cellidæ and are typically attached forms with or without prolongations of the posterior ends in the form of stalks. Such stalks may or may not contain prolongations of the neuromotor apparatus and are correspondingly either highly contractile or rigid. The two other families are represented by very few genera, as follows:

Family 1. **Spirochonidæ**, Stein.—Here the peristomial area with the adoral zone of membranelles is spirally rolled. Individuals are sessile with or without a stalk. The genera are:

1. *Chilodochona*, with stalk and with membrane about the joint of stalk.
2. *Spirochona*, joint of stalk not provided with membrane, and
3. *Spirochonopsis* (questionable).

Family 2. **Lichnophoridæ**, Bütschli.—The single genus of this family lives an ectoparasitic life usually on the eggs of marine animals (*Crepidula*, etc.). The organisms are attached to the egg by a disc at the posterior end of the ventral surface; the peristomial region is enlarged and the portion of the body between it and the attaching part becomes drawn out or very much twisted with the activity of the peristomial region bearing the mouth which may turn in any direction. One genus—*Lichnophora*.

Family 3. **Vorticellidæ**, Ehrenberg, emend Bütschli.—This is not only the largest group of the Peritrichida, but of all ciliates, the accepted classification of the group is the least satisfactory and a revision is greatly to be desired. There are two sub-families—Urceolarinæ and Vorticellinæ, the former characterized by the presence of a posterior girdle of cilia which may be transformed into an attaching disc, and by the absence of the peristomial trench which is typical of the Vorticellinæ.

Sub-family Urceolarinæ.—These are very aberrant Peritrichida and are placed here only provisionally. Particularly questionable forms are those with a complete coating of cilia (*Trichodinopsis*) or with spirally wound rows of cilia on the body and a substitution of one or two huge undulating membranes for the adoral zone of membranelles (*Hemispira*). The genera provisionally included are:

1. *Trichodinopsis*, with the surface of body covered with cilia.
2. *Hemispira*, with rows of cilia; undulating membrane replaces adoral zone.
3. *Hemispeiropsis*, no cilia; two concentric membranes replace adoral zone.
4. *Trichodina*, ectoparasitic; girdle of cilia at posterior end.
5. *Cyclochæta*, parasitic; girdle of erect bristles external to posterior cilia.

Sub-family Vorticellinæ, Bütschli.—This large and inadequately characterized group is further subdivided into three "Tribes" according to the presence of a test or house which is present in Tribe Cothurnina, absent in the other two. The latter are sepa-

rated according to the presence (Contractilia) and the absence (Acontractilia) of contractile elements in the colonial or individual stalks.

Tribe 1. *Cothurnina*, Bütschli.—Forms with tests or houses which may be sessile or borne on stalks and which are always erect, genus *Cothurnia*.

Forms with tests or houses which are recumbent or attached by the entire side, never erect. Two genera *Lagenophrys* and *Vaginicolla*. In the former the peristomial disc is raised on a neck-like base and may be lowered as a lid or operculum. In the latter the peristomial area is not thus separated from the body by a neck.

Tribe 2. *Contractilia*, Bütschli.—Here the stalks contain highly contractile myonemes which, in colonial forms may form a connected system of contractile threads throughout the colony (*Zoöthamnium*) so that the entire colony contracts; or the contractile elements may be confined to the stalks of the individuals of the colony so that the colony does not contract but the individuals composing it do (*Carchesium*). In the third genus, *Vorticella*, the individuals are not united in colonies, each is solitary and contracts on its own attached stalk.

Tribe 3. *Acontractilia*, Bütschli.—This group is the richest in number of genera but more limited in number of species than the Contractilia. The stalks when present are never provided with myonemes and are correspondingly rigid. Colonial aggregates are frequent.

The following genera are colonial:

1. *Ophrydium*, individuals are green through the presence of symbiotic forms, and embedded in jelly; the colonies are spheroidal and vary in size from $\frac{1}{2}$ inch or less, to 3 feet in diameter. Fresh water.

2. *Opercularia*, colorless colonies branching in one plane; the individuals have a peristomial lid or operculum as in *Lagenophrys* borne on a neck.

3. *Epistylis*, similar to *Opercularia* but the individuals lack the neck-like constriction, the peristomial region is similar to that of Vorticella (Fig. 210, p. 502).

The following genera are solitary:

4. *Astylozoön*, free-swimming forms without stalk; with two posterior bristles.

5. *Gerda*, creeping forms, broadest at the posterior end and ringed.

6. *Scyphidia*, cylindrical and cross-ringed forms with attaching disc.

7. *Rhabdostyla*, with short stalk, *Vorticella*-like, but non-contractile.

8. *Glossatella*, similar to *Rhabdostyla* but with enormously developed undulating membrane.

Class II. **SUCTORIA**, Bütschli.

(Also called *Tentaculifera* or *Acinetaria*).

In one interesting genus of the Suctoria—*Hypocoma*—the ventral surface bears a coating of permanent cilia (Chatton regards this form as an aberrant holotrich see p. 373). In all other genera cilia are confined to the buds or embryos during their free-swimming stages and are lost with the acquisition of tentacles and development of the attaching disc or the stalk. The individuals of the group are characteristically sessile and are attached to foreign objects usually by stalks which are short or long, slender or clumsy, but invariably rigid. Floating or suspended forms are exceptional (*Sphærophrya*). Tentacles are always present (a single one in *Hypocoma*) and may be of two kinds, one suctorial, the other sharp-pointed and adapted for piercing (Ephelotidæ, Fig. 163, p. 372). The general form of the body is highly varied, sometimes spherical, spheroidal or ellipsoidal; sometimes tetrahedral, occasionally branched and ramifying. Houses or tests are frequent. The tentacles may be distributed all over the body or may be confined to limited regions. Reproduction occurs by equal division or by budding, the latter being the more characteristic method. Budding may be either exogenous, *i. e.*, superficial, or endogenous. In the latter case one or more buds may be formed in the protoplasm about one or more micronuclei and a part of the macronucleus (Fig. 112, p. 231); these buds develop cilia in the brood pouch covered over by the anterior cell wall, and when fully formed pass out of the birth opening as ciliated embryos. After a swarming stage of variable duration they become attached and metamorphose into the adult form, or they enter other Protozoa where as parasites they live until metamorphosis occurs.

Fertilization processes have been worked out in but few forms. In *Dendrocometes* conjugation follows the general plan of conjugation in the ciliates, but, as in the ciliate *Anoplophrya branchiarum*, there is an interchange of macronuclei as well as of micronuclei.

Classification of the Suctoria is still imperfect very little having been done in this direction since Collin's masterly monograph (1911) on these forms. We follow Collin in grouping them in eight families as follows:

Family 1. **Podophryidæ.**—In these forms the body is monaxonic with a tendency to bilateral symmetry. Tentacles are of the suctorial type only distributed over the entire body or grouped in fascicles. The cells are naked or enclosed in tests which may be either delicate and close-fitting with an almost invisible rim, or coarse, loose-fitting with a conspicuous rim. Individuals are usually free-living but may be parasitic. Reproduction is by division or by exogenous budding.

The following genera are naked:

1. *Podophrya,* normally with stalk, and attached (Fig. 91, p. 185).

2. *Sphærophrya,* without stalk, free-swimming (suspended) or parasitic.

The following genera are provided with tests:

3. *Paracineta,* with close-fitting delicate test, rim invisible.

4. *Metacineta,* with coarse test, rim distinct, tentacles in fascicles.

5. *Urnula,* with coarse test, rim distinct, tentacles 1 to 3 in number.

Family 2. **Acinetidæ.**—In these forms also the body is monaxonic tending to bilateral symmetry. Tentacles of one type only (suctorial) are present and the individuals are either naked or enclosed in cups or tests, and are with or without stalks. Reproduction is by division or by internal or endogenous budding. Frequently ectoparasitic on the gills of fresh or salt water animals, or on other Protozoa; some forms are endoparasites, and are devoid of stalks and tentacles.

The following genera are parasitic:

1. *Endosphæra,* without stalk or tentacles; endoparasitic in other Protozoa.

2. *Tachyblaston,* ectoparasitic on *Ephelota.*

3. *Pseudogemma,* ectoparasitic, stalks embedded in *Acineta* or *Paracineta.*

The following genera are free-living; attached; with or without stalk.

4. *Dactylophora,* with 12 to 15 finger-form processes each with sucker.

5. *Tokophrya,* tentacles in fascicles; body in form of inverted pyramid. Stalked.

6. *Hallezia,* tentacles in fascicles; form variable; no stalk.

7. *Acineta;* with membrane-like test without free margin; tentacles anterior.

8. *Solenophrya,* test cup-like with free margin; attached by base of cup.

9. *Periacineta;* test cup-like, attached by narrowed, stalk-like base.

10. *Acinetopsis;* cup polyhedral with stalk, 1 to 6 central tentacles.

11. *Thecacineta;* test with free margin, stalked, tentacles apical and distributed.

Family 3. **Discophryidæ.**—In these forms the pellicle is coriacious and tough as distinguished from the delicate pellicle of the preceding family which they resemble in other respects. Reproduction is by endogenous budding. The tentacles may be greatly reduced in number and variable in form, some with expansile suctorial tips, others with swollen bases.

The following genera have only one primary tentacle:

1. *Rhynchophrya,* with stalk and with 1 or 2 secondary tentacles.

2. *Rhyncheta,* no stalk, attachment by protoplasmic body, 1 tentacle (?).

The following genera have many tentacles:

3. *Thaumatophrya,* tentacles conical with enlarged bases.

4. *Discophrya,* except for coriacious membrane similar to *Acineta.*

5. *Choanophrya,* tentacle expansile at extremities for food taking.

Family 4. **Dendrosomidæ.**—The individuals included in this family are highly variable in form frequently with a creeping protoplasmic stolon and with a well marked tendency to branch; stalks are exceptional. Tentacles are of one type only (suctorial). Frequently ectoparasitic.

1. *Rhabdophrya,* body not branched, cigar- or rod-shape; with stalk.

The following genera are not stalked but the body is attached:

2. *Trichophrya* (Fig. 91, p. 185), ectoparasitic, tentacles in fascicles, no branches.

3. *Dendrosoma* (Fig. 159, p. 368), with basal stolon, erect branches bearing tentacles.

4. *Lernæophrya,* base with short unbranched processes bearing fascicled tentacles.

The following genera have no stalks and are unattached:

5. *Tetrædrophrya,* body tetrahedral in form.

6. *Staurophrya,* body with 6 processes of like character.

7. *Astrophrya,* body with 8 radiating processes each with a fascicle of tentacles.

Family 5. **Dendrocometidæ.**—These are forms with somewhat specialized processes termed "arms" which may or may not be branched. They are attached forms with tentacles of one kind only.

1. *Dendrocometes,* with branched arms, each branch with one sucker.

2. *Stylocometes;* with simple, unbranched arms.

Family 6. **Ophryodendridæ.**—In this family, with only one genus—*Ophryodendron*—an arm-like process is still further differentiated to form a retractile proboscis which bears the suctorial tentacles. Little is known of the life history.

Family 7. **Ephelotidæ.**—The only forms of Suctoria with two types of tentacles—suctorial and prehensile or piercing—are included in this family. They are naked or cup-bearing and are with or without stalks. Reproduction is by external budding. Usually parasitic on marine animals particularly hydroids. Sexual processes are unknown.

1. *Ephelota* (Fig. 111, p. 230), without test or cup, with or without stalks; usually on hydroids.

2. *Podocyathus,* with test and stalk.

Family 8. **Hypocomidæ.**—The one genus in this family, *Hypocoma*, differs from all other Suctoria in having permanent cilia and a definite ventral surface bearing one tentacle in addition to the cilia. This form if not a degenerate holotrich may be interpreted as a stage of arrested development of a ciliated embryo which has developed a suctorial tentacle and has become permanent. An earlier interpretation explained it as a transitional form between the ciliates and the Suctoria.

KEY TO GENERA OF INFUSORIA.

With simple or compound cilia during vegetative life.....Class 1. Ciliata
Adults with tentacles; cilia only during development.....Class 2. Suctoria

Class I. **CILIATA**, Bütschli.

1. Body with adoral zone of membranelles... 2
   Body without adoral zone.....Order 1. Holotrichida
2. Adoral zone winds to the left..... 3
   Adoral zone winds to the right...Order 5. Peritrichida
3. Motile organs confined to ventral surface
   Order 4. Hypotrichida
   Motile organs variously distributed.... 4
4. Body covered with cilia.....Order 2. Heterotrichida
   Cilia on body absent or much reduced
   Order 3. Oligotrichida

Order I. **HOLOTRICHIDA**, Stein.

1. Mouthless (astomatous) forms; parasitic
   Sub-order 1. Astomina
   With mouth; parasitic or free-living..... 2
2. Mouths usually closed; oral membranes absent.....Sub-order 2. Gymnostomina
   Mouths usually opened; oral membranes present.....Sub-order 3. Trichostomina

Sub-order 1. **Astomina**, Cêpêde.

1. With macronuclei and micronuclei..... 2
   Nuclei two or more but all alike..*Group 1.* (*Opalinidæ*)
2. Parasites of digestive tract; various animals
   Group 2.
   Parasites of cœlomic cavity.....*Group 3.*
   Parasites of branchial cavity, uterus, gonads, etc.....*Group 4.*

Group 1. Family Opalinidæ

1. Form cylindrical; circular in cross-section. 2
   Flattened forms; ellipsoidal in cross-section 3
2. With two similar nuclei.....Genus *Protoöpalina*
   With many similar nuclei.....Genus *Cepedea*
3. With two similar nuclei.....Genus *Zelleriella*
   With many similar nuclei.....Genus *Opalina*

Group 2. Parasites of the digestive tract

1. Macronucleus simple; bar, band, sphere, etc. . . . 2
   Macronucleus branched (*Polydora* parasite) Genus *Rhizocaryum*
2. Without anchoring apparatus or suckers, etc. . . . 3
   With spicules, suckers, spines or other apparatus. . . . 4
3. Body pyriform; canals of medusæ. . . Genus *Kofoidella*
   Body vermiform; rounded both ends Genus *Anoplophrya*
4. With attaching spine. . . . 5
   With sucker-like region; vacuole a long, lateral canal. . . . 8
5. With vestigial oesophagus (Tubifex parasite). . . . Genus *Intoschellina*
   Without traces of gullet. . . . 6
6. Anterior end obliquely truncate; spicule internal. . . . Genus *Mesnilella*
   Spicule protrudes from anterior end. . . . 7
7. Spicule a conspicuous spine (*Lumbricus* parasite Fig. 165). . . . Genus *Hoplitophrya*
   Spicule a mere external hardening. . Genus *Maupasella*
8. With two anterior spines. . . . Genus *Steinella*
   No anterior spines. . . . 9
9. With anterior sucker and neck-like constriction. . . . Genus *Discophrya*
   With anterior sucker without constriction Genus *Haptophrya*

Group 3. Cœlomic parasites

1. Without contractile vacuole; anterior rostrum. . . . Genus *Herpetophrya*
   With contractile vacuole. . . . 2
2. Vacuole single, posterior; copepod parasites Genus *Perezella*
   Numerous vacuoles; distributed; copepod blood. . . . Genus *Collinia*

Group 4. Parasites of branchial cavity, gonads, uterus, etc.

Vestigial mouth and gullet; snail uterus Genus *Protophrya*
Parasite of echinoderm gonads. . . . . Genus *Orchitophrya*

Sub-order 2. **Gymnostomina**, Bütschli.

1. Parasites; thickened cortex; narrow trench to mouth. . . . Family 1. Nicollellidæ
   Free or parasitic; no thickened cortex. . . . 2
2. Mouth terminal; usually closed. . Family 2. Enchelinidæ
   Mouth not terminal. . . . 3
3. Anterior end more or less proboscis-like Family 4. Trachelinidæ
   Anterior rounded; usually with oral basket Family 3. Chlamydodontidæ

Family 1. **Nicollellidæ**, Chatton.

1. Thick cortex not more than half ventral side. . . . Genus *Nicollella*
   Thick cortex more than half ventral side 2

2. Thick cortex extends to posterior end
Genus *Collinella*
Thick cortex on both ventral and dorsal sides.....Genus *Pycnothrix*

Family 2. **Enchelinidæ**, Ehrenberg.

1. With uniform cilia over whole or part of cell 2
With one or more girdles of conspicuous cilia..... 22
2. Axopodial processes in addition to cilia... 3
Without axopodia or other pseudopodia... 4
3. Long pseudopodia with axial filaments
Genus *Myriaphrys*
Short pseudopodia-like processes...Genus *Dactylochlamys*
4. Body with long caudal hair or hairs..... 5
Body without posterior hairs..... 7
5. Body uniformly ciliated..... 6
Cilia absent from posterior third of body
Genus *Balanitozoön*
6. House- or test-dwelling.....Genus *Pelamphora*
Without house or test (Fig. 165)...Genus *Urotricha*
7. With temporary forest of tentacles (Fig. 81, p. 154).....Genus *Actinobolus*
Without tentacles..... 8
8. Body enclosed in cuirass of plates..... 9
Body without covering plates..... 11
9. Posterior end truncate; with spines (Fig. 65, p. 128).....Genus *Coleps*
Posterior end without spines..... 10
10. Posterior end pointed.....Genus *Tiarina*
Posterior end rounded.....Genus *Plagiopogon*
11. With visible gullet and pharyngeal trichites 12
Mouth closed, without visible armature... 16
12. Mouth surrounded by circlet of long cilia 13
Mouth without special cilia..... 15
13. Body flexible, worm-like..... 14
Body rigid.....Genus *Metacystis*
14. Short row of trichocysts down ventral side
Genus *Cranotheridium*
Without trichocysts; mouth often quadrangular (Fig. 165).....Genus *Trachelocerca*
15. Elongate; knob on end of neck-like proboscis.....Genus *Trachelophyllum*
Body spheroidal or elongate; no knob (Fig. 165).....Genus *Prorodon*
16. Mouth circular..... 17
Mouth slit-like, covering most of anterior end..... 21
17. Neck-like constriction with circle of long cilia (Fig. 76, p. 148).....Genus *Lacrymaria*
Without constriction..... 18
18. Body elongate, highly metabolic (Fig. 166, p. 379).....Genus *Chœnia*
Oval to ellipsoid, neither metabolic nor rigid..... 19
19. Mouth region sharply truncated..... 20
Mouth region rounded; lips often tumid... 19a

19a. Ectoparasites on fresh water fish. . . Genus *Ichthyophthirius*
Free living, not parasitic (Fig. 166, p. 379) Genus *Holophrya*
20. Body flask-shape (Fig. 166, p. 379). Genus *Enchelys*
Body ellipsoidal. . . . . Genus *Lagynus*
21. With ventral non-ciliated adhesive disc Genus *Balantidiopsis*
Flask-shape, no adhesive disc (Fig. 90, p. 181). . . . . Genus *Spathidium*
22. Two to four small oral tentacles; girdle median (Fig. 163, p. 372). . . . . Genus *Mesodinium*
Without oral tentacles. 23
23. With one girdle of powerful cilia. 24
More than one girdle of long cilia. 25
24. Body cilia not appreciably reduced (Fig. (166, p. 379). . . . . Genus *Cyclotrichium*
Cilia reduced to small lateral area (Fig. 75, p. 146). . . . . Genus *Monodinium*
25. With two girdles of long cilia. 26
More than two girdles of cilia. . . . . Genus *Dinophrya*
26. Body cilia in addition to girdles (Fig. 75, p. 146. . . . . Genus *Askenasia*
With girdles only, no other cilia (Fig. 89, p. 180). . . . . Genus *Didinium*

Family 3. **Chlamydodontidæ**, Stein.

1. Body ellipsoid or slightly compressed. 2
Body lens-shape or ventrally flattened. . . . 3
2. Body covered with uniform cilia (Fig. 13, p. 35). . . . . Genus *Nassula*
With spiral, ciliated narrow groove. Genus *Trichospira*
3. Body lens-shape. 4
Body flattened. 5
4. Mouth anterior with corneous, curved, tube Genus *Pseudomicrothorax*
Tube similar, bundle of long cilia from mouth. . . . . Genus *Leptopharynx*
5. Body without caudal appendage. 6
Body with caudal appendage. 11
6. With small anterior tentacle; mouth questionable. . . . . Genus *Lophophorina*
Without anterior tentacle. 7
7. Mouth and trichites in anterior half of body 8
Mouth anterior (?) trichites posterior to center (Fig. 166, p. 379). . . . . Genus *Opisthodon*
8. Mouth a transverse slit across entire body Genus *Gastronauta*
Mouth circular. 9
9. Body with cross-striped band around edge Genus *Chlamydodon*
Body without cross-striped band. 10
10. Mouth on right side of anterior angle (Fig. 83, p. 158). . . . . Genus *Orthodon*
Mouth central or toward right side (Fig. 34, p. 77). . . . . Genus *Chilodon*
11. Caudal appendage a ciliated brush. . Genus *Trichopus*
Caudal appendage a spine-like process. . . . 12

12. Ventral ciliated surface very narrow Genus *Dysteria*
Ventral ciliated surface broad 13
13. Dorsal surface markedly convex.... Genus *Phascolodon*
Dorsal surface slightly arched 14
14. Ventral surface like *Chilodon,* with posterior spine ..... Genus *Dysteropsis*
Ventral surface uniformly ciliated, no spine 15
15. Cilia arranged in bands 16
Cilia in uniform rows 17
16. With dorsal keel or ridge ..... Genus *Scaphidiodon*
No dorsal keel or ridge ..... Genus *Trochilia*
17. With oblique fringe of longer cilia to mouth Genus *Onychodactylus*
Without such fringe ..... Genus *Ægyria*

Family 4. **Trachelinidæ.**

1. Proboscis single, more or less developed; with mouth 2
Proboscis double or triple; no definite mouth 7
2. Circular mouth at base of proboscis, dorsal Genus *Trachelius*
Mouth on ventral concave surface 3
3. Mouth slit-like 4
Mouth a circular pore at base of proboscis (Fig. 6, p. 28) ..... Genus *Dileptus*
4. Mouth from tip to base of well-defined proboscis (Fig. 30, p. 70) ..... Genus *Lionotus*
Proboscis rudimentary 5
5. Mouth arcuate, just below beak-like proboscis (Fig. 167) ..... Genus *Loxodes*
Mouth straight, more or less oblique 6
6. Body flask-shape or slightly compressed *Genus Amphileptus*
Body flat (Fig. 167) ..... Genus *Loxophyllum*
7. Two anterior, lateral, hollow, arms. Genus *Arachnidiopsis*
Three proboscides, each *Dileptus*-like Genus *Teutophrys*

Sub-order 3. **Trichostomina,** Ehrenberg, Stein.

1. Cilia not in zones 2
Cilia in two broad zones; with caudal tuft Family 1. Urocentridæ
2. Body with small circular or ellipsoidal peristome 3
Body with large prominent peristome 4
3. Mouth in anterior half of body.. Family 2. Chiliferidæ
Mouth in posterior half of body. Family 3. Microthoracidæ
4. One or more huge undulating membranes in peristome ..... Family 4. Pleuronemidæ
Undulating membrane in gullet. Family 5. Paramecidæ
Parasitic forms in stomach of horse and ruminants ..... Family 6. Isotrichidæ

Family 1. **Urocentridæ,** Clap. and Lach.

One genus with characters of the family (Fig. 168) ..... Genus *Urocentrum*

Family 2. **Chiliferidæ,** Bütschli.

1. Mouth terminal 2
   Mouth not terminal 3
2. Mouth slit-like, entire anterior end . Genus *Leucophrys*
   Mouth circular with lateral pocket; cup dwelling . . . . . Genus *Cyrtolophosis*
3. With caudal filament or filaments 4
   Without caudal filaments 5
4. Spiral circlet of longer cilia from mouth (Fig. 168) . . . . . Genus *Loxocephalus*
   Without spiral row of cilia . . . . . Genus *Uronema*
5. Mouth subterminal; body cigar-shape (Fig. 168) . . . . . Genus *Dallasia*
   Body ellipsoid, kidney-shape, or bean-shape 6
6. Body ellipsoid or oval 7
   Body kidney-shape or bean-shape 10
7. Mouth circular or crescentic (Fig. 168) Genus *Ophryoglena*
   Mouth oval or ellipsoid 8
8. With conspicuous pharyngeal basket Genus *Clathrostoma*
   Without pharyngeal basket 9
9. Long groove running posteriorly from mouth (Fig. 168) . . . . . Genus *Frontonia*
   Groove reduced to posterior pocket of mouth . . . . . Genus *Monochilum*
10. Body distinctly kidney-shape 11
    Body bean-shape 14
11. Mouth more or less triangular; two membranes (Fig. 168) . . . . . Genus *Glaucoma*
    Mouth with single membrane 12
12. Periplast with distinct striping, plaid-like Genus *Placus*
    Periplast without cross striping 13
13. Prominent posterior dorsal, pigmented lobe Genus *Tillina*
    Without pigmented lobe . . . . . Genus *Colpoda*
14. Peristome a long, narrow, transverse furrow . . . . . Genus *Plagiopyla*
    Peristome small 15
15. Dorsal side arched; mouth anterior . Genus *Colpidium*
    Dorsal side flat; mouth and peristome central . . . . . Genus *Chasmatostoma*

Family 3. **Microthoracidæ,** Wrzesniowski.

1. Crescentic or sickle-shape; mouth central Genus *Drepanomonas*
   Lens shape 2
2. Posterior end obliquely truncate . . . . Genus *Cinetochilum*
   Posterior end not truncate . . . . . Genus *Microthorax*

Family 4. **Pleuronemidæ,** Bütschli.

1. Cup-dwelling forms 2
   Not cup-dwelling 3
2. Cup open both ends; one anterior, one posterior bristle . . . . . Genus *Calyptotricha*
   Cup simple; peristomial lobe with long cilia Genus *Mycterothrix*

3. With one caudal filament 4
   Without caudal filament 5
4. Peristome narrow, short, body ellipsoid (Fig. 168, p. 385) . . . . . Genus *Cyclidium*
   Peristome narrow; two pseudomembranes; elongate (Fig. 170) . . . . . Genus *Lembus*
5. Free-living; peristome long and deep 6
   Commensal or ectoparasitic 9
6. With balloon or sail-like membrane 7
   One pseudomembrane of long cilia; spindle-shape . . . . . Genus *Blepharostoma*
7. Special cilia adapted for attachment 10
   Without special attaching cilia 8
8. Peristome occupies most of ventral surface (Fig. 77, p. 149) . . . . . Genus *Lembadion*
   Ellipsoidal with one sail-like membrane (Fig. 169, p. 385) . . . . . Genus *Pleuronema*
9. Kidney-shape; ectoparasitic on hydroids Genus *Pleurocoptes*
   Flat; four or five lines of cilia; commensal on Gammarus . . . . . Genus *Larvulina*
10. Ellipsoidal; attaching cilia posterior end . . 11
    Spherical; peristome turned up; five rows cilia . . . . . Genus *Hemispira*
    Urn-shape; no cilia; two concentric membranes . . . . . Genus *Hemispiropsis*
11. Pseudomembrane spiral at anterior end. Genus *Boveria*
    Membrane lateral, not spirally wound Genus *Ancistrum*

Family 5. **Paramecidæ**, Bütschli.

One genus with family characteristics (Fig. 170, p. 385) . . . . . Genus *Paramecium*

Family 6. **Isotrichidæ**, Bütschli.

Parasitic in mantle fluids of bivalves . . Genus *Conchophthirus*
Parasites of fore-stomach of ruminants . Genus *Isotricha*
Genus *Dasytricha*

Order II. **HETEROTRICHIDA**, Stein.

1. Adoral zone not parallel with main body axis 2
   Adoral zone parallel with main body axis 3
2. Body ellipsoidal or trumpet-shape Family 1. Stentoridæ
   Medusa-shape; with manubrium-like posterior end . . . . . Family 4. Gyrocorycidæ
   Discoid; laterally compressed . . . Family 5. Ctenostomidæ
3. Peristome wide, insunk, and usually deep Family 2. Bursaridæ
   Peristome narrow and long . . . . . Family 3. Plagiotomidæ

Family 1. **Stentoridæ**, Stein.

1. Body not trumpet-shape 2
   Body when expanded, trumpet-shape (Fig. 74, p. 145) . . . . . Genus *Stentor*
2. Frontal field drawn out into long arms (Fig. 84, p. 160) . . . . . Genus *Folliculina*
   Frontal field not drawn out 3

3. Pear-shape; base widest; with pigment spot Genus *Fabrea*
Compressed; frontal field oblique (Fig. 56, p. 107) ..... Genus *Climacostomum*

Family 2. **Bursaridæ**, Stein.

1. Free-living 2
Parasitic; peristome to center of body Genus *Balantidium*
2. Frontal field deeply insunk; sac-like 3
Body worm-like, very contractile (Fig. 171) Genus *Condylostoma*
3. Walls of sac with cortical trichocysts Genus *Thylakidium*
Walls of sac without trichocysts (Fig. 84, p.160) ..... Genus *Bursaria*

Family 3. **Plagiotomidæ**, Clap. and Lach.

1. Free-living 2
Internal parasites 6
2. Laterally compressed 3
Not compressed 4
3. Peristome oblique with spiral gullet Genus *Helicostoma*
Peristome oblique, straight; usually pink (Fig. 174) ..... Genus *Blepharisma*
Adoral zone spirally wound from end to end Genus *Phacodinium*
4. Body long, narrow; peristome narrow, straight (Fig. 30, p. 70) ..... Genus *Spirostomum*
Body ellipsoidal, not contractile 5
5. Body straight, two crescentic oral lips Genus *Porpostoma*
Body twisted anteriorly; no crescentic lips (Fig. 171) ..... Genus *Metopus*
6. Form oval to bean-shape (Fig. 171) Genus *Nyctotherus*
Elongate; pointed anteriorly; truncate below ..... Genus *Plagiotoma*

Family 4. **Gyrocorycidæ**, Stein.

Anterior end rounded; umbrella-like (Fig. 174) Genus *Cœnomorpha*
Anterior end flattened; two bands of cilia Genus *Trochella*

Family 5. **Ctenostomidæ**, Lauterborn.

1. Anterior end with ventrally pointed spine 2
Without ventrally directed anterior spine 3
2. With two unequal spines on the right side Genus *Discomorpha*
Seven or eight posterior spines; none on sides ..... Genus *Saprodinium*
3. Posterior end with bisected incut ... Genus *Pelodinium*
Posterior drawn out into seven to eight blunt processes ..... Genus *Epalxis*

Order III. **OLIGOTRICHIDA**, Bütschli.

1. Parasitic in stomach ..... Family 3. Ophryoscolecidæ
Free-living 2
2. Without house or test ..... Family 1. Halteriidæ
With house or test (mainly marine). Family 2. Tintinnidæ

Family 1. **Halteriidæ,** Clap. and Lach.

1. Spheroidal; central girdle of bristle-like cilia.....Genus *Halteria*
   Spheroidal or urn-shape; no girdle of cilia 2
2. Periplast delicate..... 3
   Posterior periplast hardened and test-like Genus *Labœa*
3. With distinct tail-like appendage...Genus *Tontonia*
   Without caudal appendage.....Genus *Strombidium*

Family 2. **Tintinnidæ,** Clap. and Lach. (Mainly after Jorgensen, 1899).

1. Test soft and gelatinous.....Genus *Tintinnidium*
   Test rigid, chitinous..... 2
2. Test open at both ends.....Genus *Tintinnus*
   Test open at anterior end only..... 3
3. Test with anterior decorations..... 4
   Test plain without anterior trimmings.... 5
4. Anterior perforated by large openings Genus *Dictyocysta*
   Anterior openings absent or feeble..Genus *Codonella*
5. Test wall double with alveoli.....Genus *Cyttarocyclis*
   Test wall double without alveoli....Genus *Undella*
   Test wall single, simple..... 6
6. Test ornamented by pleats or sculpturing Genus *Ptychocyclis*
   Test with adherent foreign particles (Fig. 174, p. 391).....Genus *Tintinnopsis*
   Test without foreign bodies or ornaments Genus *Amphorella*

Family 3. **Ophryoscolecidæ,** Stein.

1. With one circle of membranelles..... 2
   With two circles of membranelles..... 3
2. Two posterior bundles of cilia (*Caudalia*) Genus *Cycloposthium*
   No caudal cilia, long caudal spine..Genus *Entodinium*
   Four incomplete girdles of long cilia Genus *Troglodytella*
3. Dorsal circlet includes more than half the body.....Genus *Ophryoscolex*
   Dorsal circlet includes less than half the body (Fig. 2, p. 20).....Genus *Diplodinium*

Order IV. **HYPOTRICHIDA,** Stein.

1. Ventral surface with uniform cilia; no cirri Family 1. Peritromidæ
   At least anal cirri on ventral surface..... 2
2. Except anals, no ventral cirri posterior to mouth.....Family 2. Urostylidæ
   With posterior ventral cirri..... 3
3. With cilia in addition to cirri..... 4
   Cilia entirely replaced by cirri..... 5
4. Five to eight frontal cirri; cilia in one or more rows.....Family 3. Pleurotrichidæ
   Frontal and ventral cirri reduced; no cilia Family 4. Psilotrichidæ
5. With lateral, ventral, frontal and anal cirri Family 5. Euplotidæ
   No lateral; variable frontals, ventrals, and anals.....Family 6. Aspidiscidæ

Family 1. **Peritromidæ**, Stein.

One genus with the characters of the family
Genus *Peritromus*

Family 2. **Urostylidæ.**

1. Ectoparasitic on *Hydra*, etc. . . . . Genus *Kerona*
   Free-living forms, usually bottom feeders . . 2
2. No differentiated frontal cirri 6
   With from three to several frontal cirri 6
3. Ventral rows of cilia straight 4
   Ventral cilia rows spiral 5
4. With neck and tail; six to nine rows of cilia (Fig. 174) . . . . . Genus *Epiclintes*
   No neck or tail; two marginal; two ventral rows cilia . . . . . Genus *Holosticha*
5. Peristome long; often tube-dwelling (Fig. 173, p. 390) . . . . . Genus *Stichotricha*
   Peristome only half the length of proboscis. Genus *Sparotricha*
6. Ventral surface covered with fine cilia Genus *Trichogaster*
   Ventral surface with few rows of cilia 7
7. No anal cirri; two to four rows of ventral cilia 8
   With anal cirri 9
8. No long caudal cilia or bristles (Fig. 1, frontispiece) . . . . . Genus *Uroleptus*
   With three caudal cilia or bristles . . Genus *Strongylidium*
9. Two rows marginal; two to three rows ventral cilia (Fig. 175) . . . . . Genus *Amphisia*
   Two rows marginal; five to seven rows ventral cilia . . . . . Genus *Urostyla*

Family 3. **Pleurotrichidæ**, Bütschli.

1. Posterior end drawn out as a distinct tail Genus *Urosoma*
   Posterior end not tail-like 2
2. Peristome very narrow 3
   Peristome broad, triangular 4
3. Five anterior ray-like spikes (membranelles) . . . . . Genus *Actinotricha*
   No ray-like spikes; three caudal cirri Genus *Gonostomum*
4. Body very flexible; tail bristles short, if any 5
   Body slightly flexible or rigid 6
5. Five to six frontal; irregular rows of ventral cirri . . . . . Genus *Gastrostyla*
   Eight frontal, five ventral, undeveloped caudal cirri (Fig. 175) . . . . . Genus *Oxytricha*
6. No caudals; marginal row of cilia unbroken posteriorly 7
   Three caudals; marginal row broken posteriorly . . . . . Genus *Stylonychia*
7. Row of anal cirri broken; two nearer posterior end (Fig. 176) . . . . . Genus *Pleurotricha*
   Anal cirri form a continuous line 8
8. Large; anterior and posterior ends truncate (Fig. 172, p.) 389 . . . . . Genus *Onychodromus*
   Small; oval to ellipsoidal (Fig. 175) . Genus *Histrio*

Family 4. **Psilotrichidæ**, Bütschli.
Heavy bristle-like marginal; one row ventral cirri.....Genus *Balladina*
Irregularly scattered cirri (Fig. 176)...Genus *Psilotricha*
Family 5. **Euplotidæ**, Ehrenberg, Stein.
1. No special anal or frontal cirri; seven to nine posteriors (Fig. 107, p. 225).Genus *Uronychia*
With five large anal cirri..... 2
2. Three posterior giant cirri; seven to eight frontal-ventral cirri.....Genus *Diophrys*
No giant cirri; more than eight ventral cirri 3
3. Eleven cirri on left margin; eleven frontal-ventrals.....Genus *Certesia*
Four cirri on posterior margin; nine to ten frontal-ventrals.....Genus *Euplotes*
Family 6. **Aspidiscidæ**, Stein.
The peristome begins at the anterior end Genus *Aspidisca*
The peristome begins at center of the left edge (Fig. 80, p. 152).....Genus *Onychaspis*

Order V. **PERITRICHIDA**, Stein.

1. With spirally rolled peristome...Family 1. Spirochonidæ
Peristome not spirally rolled..... 2
2. Ectoparasites; attachment posterior end, flexible.....Family 2. Lichnophoridæ
Not parasitic, posterior end not flexible Family 3. Vorticellidæ

Family 1. **Spirochonidæ**, Stein.
Stalked, membrane about joint of stalk Genus *Chilodochona*
With or without stalk, joint without membrane.....Genus *Spirochona*
Family 2. **Lichnophoridæ**, Bütschli.
One genus with characters of the Family Genus *Lichnophora*
Family 3. **Vorticellidæ**, Ehrenberg, emend Bütschli.
No peristomial trench, attaching disc ciliated Sub-family 1. Urceolarinæ
With peristomial trench, posterior cilia temporary.....Sub-family 2. Vorticellinæ

Sub-family 1. Urceolarinæ. Bütschli.
1. Surface of body covered with cilia..Genus *Trichodinopsis*
Surface not covered with cilia..... 2
2. Erect bristles outside posterior girdle of cilia.....Genus *Cyclochæta*
No bristles in addition to posterior cilia Genus *Trichodina*
Sub-family Vorticellinæ. Bütschli.
Cells with contractile stalks.....Tribe Contractilia
Cells without contractile stalks.....Tribe Acontractilia
Cells in tests or houses.....Tribe Cothurnina

Tribe 1. *Contractilia*, Bütschli.

Note.—Individuals are often detached from their stalks under laboratory conditions and become free-swimming; such detached forms always have a posterior girdle of cilia, one genus—*Opisthonecta*—has them permanently.

1. Solitary; a single highly contractile stalk ..... Genus *Vorticella*
   Colonial; colonies dichotomously branched 2
2. Individual stalks contract separately; not connected ..... Genus *Carchesium*
   Entire colony contracts; stalk threads connected ..... Genus *Zoöthamnium*

Tribe 2. *Acontractilia*, Bütschli.

1. Colonial forms; colonies often enormous 2
   Solitary forms 4
2. Individuals of huge colony embedded in jelly ..... Genus *Ophrydium*
   Individuals not embedded in jelly 3
3. Peristome disc not stalked; feather-like colonies (Fig. 210, p. 502) ..... Genus *Epistylis*
   Peristome disc stalked; feather-like colonies ..... Genus *Opercularia*
4. Individuals free-swimming or creeping 5
   Individuals attached 6
5. No stalk; posterior cilia girdle permanent ..... Genus *Opisthonecta*
   Vorticella-like; two posterior bristles; no stalk ..... Genus *Astylozoön*
   Posterior end broad, ringed near base, no bristles ..... Genus *Gerda*
6. Solitary, in delicate cup; peristome cup-like ..... Genus *Ophrydiopsis*
   Cylindrical, with attaching disc; cross-ringed ..... Genus *Scyphidia*
   Vorticella-like; with or without short stalk 7
7. Undulating membrane enormously developed ..... Genus *Glossatella*
   Undulating membrane inconspicuous; stalk short ..... Genus *Rhabdostyla*

Tribe 3. *Cothurnina*, Bütschli.

1. Upright; attachment posterior; with or without stalk ..... Genus *Cothurnia*
   Attachment lateral or lengthwise; recumbent 2
2. Peristome disc with stalk, operculum-like ..... Genus *Lagenophrys*
   Peristome disc not stalked ..... Genus *Vaginicola*

Class II. **SUCTORIA**, Bütschli.

1. Cilia absent except on embryos 2
   Body permanently ciliated ..... Family Hypocomidæ
2. Suctorial tentacles alone present 3
   Prehensile tentacles in addition to suctorial ..... Family Ephelotidæ

3. Body not bilaterally symmetrical; irregular or branched 4
   Body monaxial; more or less bilateral 6
4. Without "proboscis" or special "arms" Family DENDROSOMIDÆ
   With retractile proboscis or special "arms" 5
5. With retractile proboscis . . . . . Family OPHRYODENDRIDÆ
   With special, tentacle-bearing "arms" Family DENDROCOMETIDÆ
6. Reproduction by internal budding 7
   Reproduction by external budding . Family PODOPHRYIDÆ
7. Pellicle delicate . . . . . Family ACINETIDÆ
   Pellicle tough, coriacious . . . . . Family DISCOPHRYIDÆ

Family **Actinetidæ.**

1. Internal parasites (no stalk; no tentacles) Genus *Endosphæra*
   External parasites or free-living 2
2. Parasitic on other suctoria 3
   Not parasitic on suctoria; or free-living . . . 4
3. Stalk embedded in Acineta or Paracineta Genus *Pseudogemma*
   Parasitic on Ephelota . . . . . Genus *Tachyblaston*
4. Twelve to fifteen finger-form processes, each with sucker . . . . . Genus *Dactylophora*
   Without finger-form processes 5
5. Test or cup absent; tentacles in fascicles . 6
   Test or cup present 7
6. Body pyramidal, with stalk (Fig. 112, p. 231) . . . . . Genus *Tokophrya*
   Form variable; no stalk . . . . . Genus *Hallezia*
7. Test without free margin, membrane-like (Fig. 91, p. 185) . . . . . Genus *Acineta*
   Test cup-like, with free rim or margin 8
8. No definite stalk; test attached by base . . . 9
   Test attached by definite stalk 10
9. Cup attached by entire base . . . . . Genus *Solenophrya*
   Base of cup narrowed, almost stalk-like Genus *Periacineta*
10. Cup polyhedral; one to six central tentacles Genus *Acinetopsis*
    Cup not polyhedral; distributed apical tentacles . . . . . Genus *Thecacineta*

Family **Discophryidæ.**

1. One primary tentacle; with or without secondaries 2
   With many tentacles 3
2. With stalk . . . . . Genus *Rhynchophrya*
   No stalk; attachment by protoplasmic body . . . . . Genus *Rhyncheta*
3. Suctorial tentacles conical, with enlarged bases . . . . . Genus *Thaumatophrya*
   Tentacles uniform in diameter 4
4. Tentacles expansile at extremities for food-taking . . . . . Genus *Choanophrya*
   Tentacles not expansile . . . . . Genus *Discophrya*

Family **Dendrosomidæ.**

1. Forms with stalk 2
   Forms without stalk 3
2. Body much branched, finger-like . . . Genus *Dendrocometes*
   Body bar-like, not digitate . . . . . Genus *Rhabdophrya*
3. Body attached 4
   Body free 6
4. Body bilateral or slightly asymmetrical (Fig. 91, p. 185) . . . . . Genus *Trichophrya*
   Body flat 5
5. With basal stolon; branches erect; often second branches (Fig. 159, p. 368) Genus *Dendrosoma*
   No stolon; short unbranched processes, fascicled tentacles . . . . . Genus *Lernæophrya*
6. Body tetrahedral . . . . . Genus *Tetrædrophrya*
   Body polyhedral 7
7. With six similar protuberances . . . . . Genus *Staurophrya*
   With eight radiate processes, each with a fascicle . . . . . Genus *Astrophrya*

Family **Dendrocometidæ.**

Arms branched, each branch with one sucker Genus *Dendrocometes*
Arms not branched . . . . . Genus *Stylocometes*

Family **Ophryodendridæ.**

One genus only . . . . . Genus *Ophryodendron*

Family **Podophryidæ.**

1. Without test or cup 2
   With test or cup 3
2. Normally with stalk, attached (Fig. 91, p. 185) . . . . . Genus *Podophrya*
   Free-swimming or parasitic . . . . . Genus *Sphærophrya*
3. Cup close-fitting, no visible rim . . . . Genus *Paracineta*
   Cup not close-fitting, rim visible 4
4. Tentacles numerous; in fascicles (Fig. 91, p. 185) . . . . . Genus *Metacinata*
   Tentacles scarce; one to three . . . . . Genus *Urnula*

Family **Ephelotidæ.**

No test or cup; with or without stalk (Fig. 111, p. 230) . . . . . Genus *Ephelota*
With cup and stalk . . . . . Genus *Podocyathus*

Family **Hypocomidæ.**

Only one genus, with family characters Genus *Hypocoma*

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CHAPTER IX.

SPECIAL MORPHOLOGY AND TAXONOMY OF THE SPOROZOA.

Forms adapted to a parasitic mode of life are found in every main group of the Protozoa and several highly pernicious human diseases such as dysentery, Leishmaniases, and African sleeping sickness are due to them. Such forms, however, may be regarded as having arisen as casual parasites which owe their parasitic mode of life to their original power to resist the digestive fluids and other conditions of the animal body. Such adaptations are always possible in normally free-living microörganisms subject to ingestion with food and drink.

Sporozoa are obligatory parasites and free-living forms are unknown. Practically all kinds of animals are subject to invasion by one type or other and adaptations are manifold and varied in response to the necessary and often highly specialized conditions of their existence. As with parasites generally, a necessary adaptation for the maintenance of species is the power of prolific multiplication. This is realized by the universal method of reproduction by spore formation to which the group owes its name. Such sporulation may occur as multiple reproduction of vegetative individuals without sexual processes or it may follow as a result of fertilization. Asexual and sexual processes give rise to typical alternation of generations in the majority of forms and complicated life histories result.

Asexual reproduction may occur by equal division (*e. g.*, *Ophryocystis*, *Babesia*, etc.); by budding which may be exogenous (Myxosporidia) or endogenous (as in the gregarines *Schizocystis* and *Eleutheroschizon*), or by multiple division (Coccidiomorpha). Reproduction following fertilization always involves the formation and the permanent fusion of gametes. These may be isogamous or anisogamous and dimorphic gametes as different as are eggs and spermatozoa of the Metazoa are characteristic of the Coccidia and Hæmosporidia. Sexual processes of peculiar type and regarded as self fertilization or autogamy are characteristic of the Cnidosporidia where such processes with resulting sporulation, take place in endogenous buds.

Sporulation following fertilization in the majority of forms, involves adaptations for preservation of the species during exposure to the conditions external to the definitive host. Such spores are

protected against drought and other external conditions by resistant spore membranes or capsules which are opened or dissolved only in the digestive tract of a new host. In the majority of cases such new hosts are individuals of the same species and infection is brought about by eating contaminated food. In many forms, however, the life cycle involves a change of hosts the metagamic spores developing in one type of animal and the sexual phases of the parasite developing in another type belonging to an entirely different group of the animal kingdom. Thus vegetative stages of the genus *Aggregata* develop in the crab (*Portunus depurator*) and the sexual stages in the cephalopod (*Sepia officinalis*); vegetative stages of the malaria organisms, *Plasmodium*, *Laverania*, and *Hæmoproteus* develop in the blood of man or birds and the sexual stages in the mosquito. In these blood infesting Sporozoa a further adaptation is noted in the loss of the characteristic capsules of the metagamic spores which are inoculated with the bite of the mosquito directly into the blood. Spore capsules here would make an impossible barrier to development and such forms are obligatory parasites in all phases of their life history. In some cases parasites reach the blood by way of the digestive tract and infection is contaminative. The rat parasite *Hæmogregarina* (*Hepatozoön*) *perniciosa* (according to Miller, 1908) forms its metagamic spores in the rat mite (*Lelaps echidninus*). Such infected mites are eaten by the rat and the spores develop in liver cells through some agametic generations, the agamic spores finally entering the blood where they are taken up by leukocytes in which the parasites encyst. Such encysted spores are taken with the blood into the gut of the flea where sexual phases take place and metagamic spores are formed (Fig. 177).

Although wide differences exist in the life cycles of the various kinds of parasites included in the Sporozoa there is a sufficient general resemblance in all to indicate a fundamental common type. A special nomenclature has grown up for these parasitic forms which is generally limited to the Sporozoa although there is no reason why the terms, if useful should not be applied with equal right to the stages in the life history of free-living forms. We shall use here the terminology suggested by Hartmann as modified by Doflein with such changes as will make the terms applicable to both free-living and parasitic forms. These terms are:

1. *Sporozoite.* A spore or germ produced as a result of fertilization.

2. *Agamont.* An asexual individual reproducing without fertilization (also called a schizont, or a trophozoite).

3. *Schizontocyte.* A special form of agamont which breaks up into a number of agamete-forming centers by multiple division.

4. *Agamogony* (schizogony). Asexual or agamic reproduction by equal, unequal or multiple division.

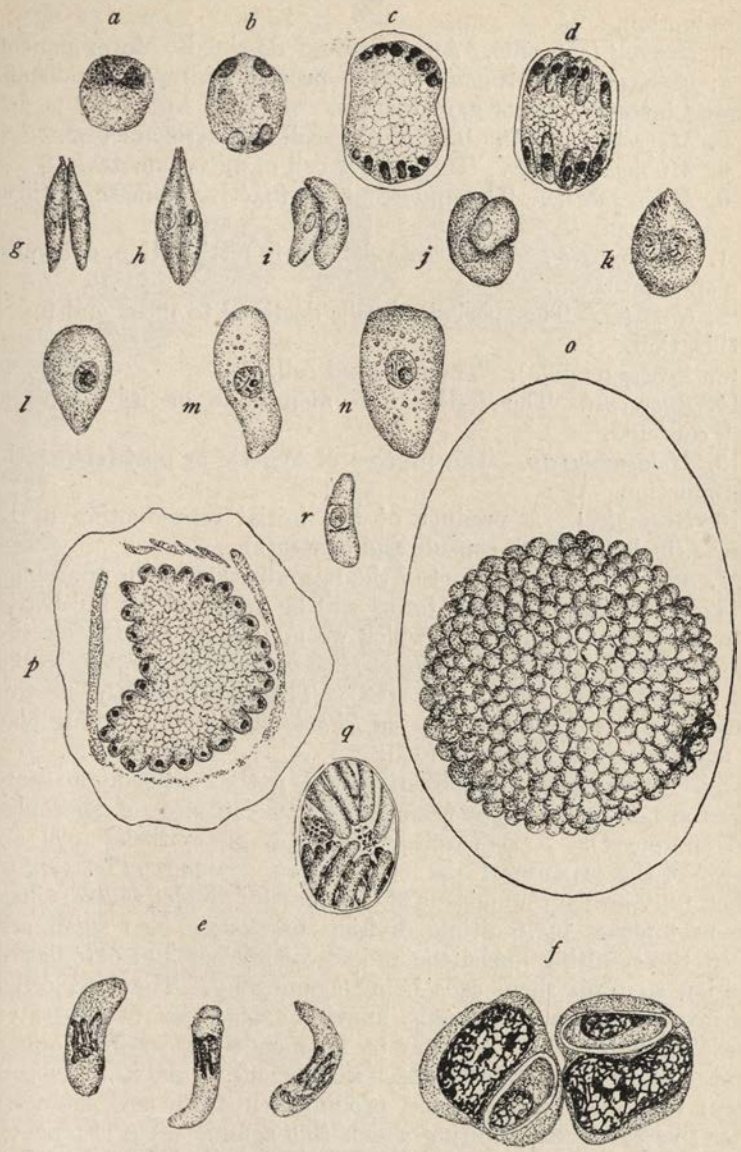

FIG. 177.—*Hæmogregarina perniciosa*, *a*, hæmogregarine parasite of the rat *a* to *d*, development of the agamont in the liver cells of the rat; *e*, free parasites in the blood; *f*, encysted parasites in the leukocytes; *g*, to *k*, stages of fusion of the gametes in the mite; *l*. to *n*, development of the zygote; *o*, sporocyst with sporoblast buds covering the surface; *p*, section of the same; *q*, older sporoblast with sporozoites. (From Calkins after Miller.)

5. *Agamete* (merozoite). An agamic spore or product of asexual reproduction.

6. *Gamont* (sporont). An individual destined to form gametes.

7. *Gamogony* (sporogony). The process of reproduction of a gamont into gametes or gametocytes.

8. *Macrogametocyte.* The mother-cell of macrogametes.

9. *Microgametocyte.* The mother cell of microgametes.

10. *Macrogamete.* The quiescent or inactive gamete fertilized by a microgamete.

11. *Microgamete.* The motile element in fertilization, equivalent to a spermatozoön.

12. *Gametes.* The specialized cells destined to meet and fuse in fertilization.

13. *Zygote* (copula). The fertilized cell.

14. *Sporocyst.* The fertilization membrane or its equivalent with contents.

15. *Metagamogony.* The process of zygotic or post-fertilization reproduction.

16. *Sporoblast.* A product of the initial reproduction of the zygote (including both capsule and contents).

17. *Sporozoite.* A product of the reproduction of the sporoblast.

The significance of these terms will be apparent by illustration with a concrete example for which we may again use the classical case of the life history of *Eimeria* (*Coccidium*) *schubergi* as worked out by Schaudinn, 1900 (Fig. 178). This is a common intestinal parasite of the familiar centipede *Lithobius,* infection taking place by feeding on contaminated food.

Under the action of the digestive fluids in the centipede the sporozoites are liberated from their protective capsules (of sporoblast and sporocyst). A sporozoite penetrates an epithelial cell and grows at the expense of the cell into an agamont (Fig. 178, *a*). When fully grown the nucleus of the parasite divides several times; the protoplasm by multiple division breaks up into small cells about the resulting nuclei the process of nuclear and cytoplasmic division to form these cells being agamogony. The host cell is destroyed and the young cells, known as agametes, are liberated. These agametes make their way by independent gregariform movement to other epithelial cells which they penetrate and in which they repeat the entire agamic cycle, producing in turn, new agametes. After five or six days during which this agamic cycle is repeated resulting in multiple infection of the epithelium, the agametes develop into gamonts or prosexual individuals. Some become large, food-stored cells which, after "maturation" processes form macrogametes directly (*e, f, g*). Others form large cells with clear protoplasm—microgametocytes—which, after repeated nuclear divisions, give rise to a multitude of microgametes, the process being a

form of gamogony. Each microgamete is provided with two flagella by means of which it moves about in the intestinal fluids until it comes in contact with a macrogamete (*h*, *i*, *j*, *s*). The gametes fuse, a macrogamete being fertilized by a single microgamete (*g*). The fertilized cell resulting from this fusion is the zygote in which the pronuclei fuse. The fertilization nucleus then

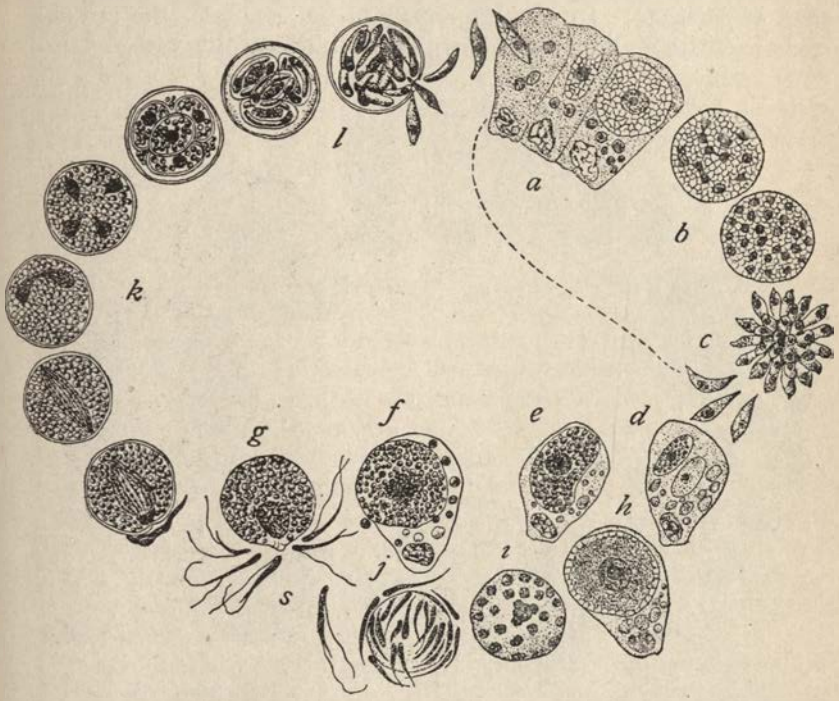

FIG. 178.—*Eimeria schubergi*. Sporozoites penetrate epithelial cells and grow into adult intracellular parasites (*a*). When mature, the nucleus divides repeatedly (*b*), and each of its subdivisions becomes the nucleus of an agamete (*c*). These enter new epithelial cells and the cycle is repeated many times. After five or six days of incubation, the agametes develop into gamonts; some are large and stored with yolk material (*d*, *e*, *f*), others have nuclei which fragment into chromidia which become the nuclei of microgametes (*d*, *h*, *i*, *j*). A macrogamete is fertilized by one microgamete (*g*) and the zygote forms a sporocyst (*k*). This forms four sporoblasts, each with two sporozoites (*l*). (After Schaudinn.)

divides and the two products divide again before the protoplasm divides into four parts, one about each of the nuclei. This process, or metagamogony, results in the formation of four sporoblasts within the sporocyst and each sporoblast has its own individual protective capsule (*l*). The nucleus of each sporoblast then divides and two independent cells are formed in each sporoblast. These independent cells are the sporozoites. To recapitulate: Sporozoites

come from sporoblasts; sporoblasts from sporocysts; sporocysts from zygotes; zygotes from fusion of gametes; gametes from gametocytes, these from gamonts; gamonts from agametes; agametes from agamonts and agamonts, originally, from sporozoites.

There are thus two complete cycles in the life history of a typical sporozoön, an asexual and a sexual cycle. There are many variations in different types and few life cycles conform exactly with that of *Eimeria*. In the Eugregarines for example, the asexual cycle is entirely eliminated, the sporozoite developing directly into

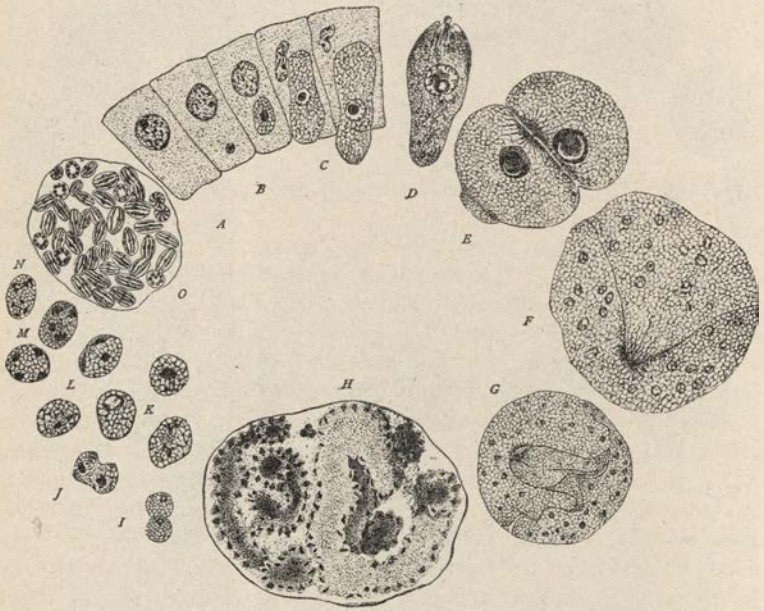

FIG. 179.—*Lankesteria ascidiæ*. Young sporozoites enter epithelial cells (*A*, *B*, *C*) and grow directly into gamonts (*D*); two of these unite in pseudoconjugation (*E*), and each forms gametes after repeated nuclear divisions (*F*, *G*, *H*). The gametes fuse two by two (*I*, *J*, *K*,) and the zygotes undergo three metagamic divisions, forming eight sporozoites (*L* to *O*). The parent cells degenerate and the sporocysts are filled with sporoblasts, each with eight sporozoites. (After Siedlecki.)

a gametocyte. In gregarines also we find a curious process which recalls the phenomenon of conjugation in the Ciliata, but which in *Adelea* is very similar to fertilization in *Vorticella*. It is termed pseudoconjugation. Two individuals come together side by side or end to end and an envelope is secreted which encloses both individuals. This envelope becomes the sporocyst membrane. Each individual now forms a large number of gametes and those from one individual fuse with the gametes from the other individual and a multitude of zygotes is formed. The actual fertilization membrane

becomes the sporoblast capsule and the zygotes divide at once to form sporozoites (Fig. 179).

Other variations will appear in the discussion of the different groups of Sporozoa as given in the following classification, in which, following the majority of students of the Protozoa, we divide the group into two classes—Telosporidia and Neosporidia—as outlined originally by Schaudinn (1900). The two groups have little in common besides the mode of life of parasites. The Class Telosporidia includes those forms in which the life of the individual comes to an end with sporulation. The Class Neosporidia includes those forms in which sporulation occurs in internal buds during the vegetative activity of the individual, sporoblasts being carried about by the still active parent cell.

Class I. **TELOSPORIDIA**, Schaudinn.

Telosporidia are Sporozoa which are invariably intracellular parasites during some phase of the life cycle. A new host is infected by contamination or by inoculation and the young germ—a sporozoite—enters some cell element, an epithelial cell if the parasite is one of the Coccidia, a blood element either blood corpuscle or blood cell if it is one of the Hæmosporidia. The adult forms of Gregarinida are invariably extracellular or lumen-dwelling parasites, young, growing stages alone being intracellular. Adult forms of Coccidiomorpha are persistent intracellular parasites throughout young, adult, and reproductive phases. Although some exceptional cases occur in both groups, these are essential differences between the two sub-classes Gregarinida and Coccidiomorpha. All are typically uninucleate in the adult phase.

Reproduction occurs either by agamogony or gamogony, the latter involving fertilization. In one Order of the Gregarinida, the Eugregarinida, the sporozoite grows directly into a gamont and asexual reproduction is unknown. In a second Order the Schizogregarinida, agamogony occurs either by equal division, internal budding, or by multiple division. In Coccidiomorpha alternation of generations is the rule and change of hosts is frequent. Multiple division is practically universal.

In both sub-classes the zygote undergoes metagamic divisions. In Gregarinida and in Hæmosporidia amongst the Coccidiomorpha, the sporozoites are formed directly by divisions of the zygote; in Coccidia the zygote divides into sporoblasts or sporozoite-forming cells. In all cases except in Hæmosporidia the sporozoites formed in each such sporoblast, are enclosed in a special capsule by which the young organisms are protected against external conditions. Hæmosporidia are obligatory parasites in one host or other throughout the entire life cycle otherwise they perish.

Except for the main groups which will probably persist, the classification of Telosporidia is not entirely satisfactory. We follow Doflein in the main, making only such changes as are necessary in raising Sporozoa to the grade of a sub-phylum instead of a class. Here also, we include in an Appendix a group of forms which have been sifted out of other groups of Protozoa or Bacteria, and included with the Sporozoa largely because there seems to be no other place to put them.

Sub-class I. GREGARINIDA.

The gregarines are typically cœlozoic or lumen-dwelling parasites of the invertebrates particularly of annelids and arthropods. They vary in size from 10 μ to 16 mm. (*Porospora gigantea*) and are prone to collect in masses in the intestine, a gregarious habit from which the name of the group is derived. Although saprozoic or osmotic in nutrition they apparently do very little if any damage to the host organism, differing in this respect from the intracellular Coccidiomorpha. The most frequent site of parasitism is the digestive tract and the glands opening into it (*e. g., Malpighian tubules*) but the sporozoites of some forms penetrate the wall of the gut and enter the body cavity where they form cysts on the cœlomic side of the intestinal wall or develop as free forms in the lumen of the seminal vesicles (Monocystidæ) or of other parts of the body cavity.

Gregarines are widely varied in form as well as in size but so far as the present accounts go they are similar in their protoplasmic make-up. A peripheral outer layer of lifeless material forms the epicyte which is equivalent to the pellicle or periplast of other Protozoa. This is secreted by the ectoplasm and is frequently drawn out into attaching organs in the form of filaments, hooks, anchors and knobs. The outer surface is often definitely ribbed, the ribs running longitudinally from end to end of the body. The furrows between the ribs are filled with a gelatinous material derived from a second layer also lifeless, of the cortex and termed by Schewiakoff the gelatinous layer. Movement of gregarines according to Schewiakoff is due to the secretion from the ectoplasm of this gelatinous material which collects from the furrows at the posterior end, hardens, and forms a resistant column against which continued secretion pushes the organism forward. The third zone of the body wall is formed by the living ectoplasm, which with the possible exception of *Stomatophora coronata* described by Hesse (1909) as possessing a mouth, peristome and cell anus, forms an unbroken, living, protoplasmic membrane. The endoplasm, or fourth zone finally, forms the bulk of the organism and contains the single nucleus usually provided with a large endosome. Paraglycogen, volutin granules and other products of living activity make the endoplasm dense and homogenous so that it appears white by reflected

and black by transmitted light. Crystals of protein-like substance are present in many cases, also crystals which have been identified as calcium oxalate. Between endoplasm and ectoplasm, finally, a system of myonemes may be found in some cases. According to Schneider these form a plexus or network of fibrils around the body; according to Doflein they form transverse rings about the cell and in longitudinal sections can be detected only as minute circular granules. Crawley, in opposition to Schewiakoff, interprets the movement of gregarines as due to these circular myoneme-like fibrils, the organism utilizing them very much as a snake uses its ribs. Some forms, notably the Monocystidæ, may be highly metabolic; others move steadily in one direction a characteristic mode of progression which has given rise to the term gregariform movement.

Motile forms are limited to the free types in the digestive tract or body cavity. Quiescent forms are usually attached to some epithelial cell by a portion of the gregarine known as the epimerite. This is a differentiation of the periplast frequently called the epicyte which in different species has characteristic and varied forms with specialized attaching processes in the form of hooks, anchors, filaments, etc. They are formed only by the polycystid gregarines or those with more than one chamber (monocystids). The epimerite is readily discarded and left in the host cell while the adult organism a gamont, lies free in the cavity of the organ. In these forms the body is divided by a transverse septum which is formed by an ingrowth of the ectoplasm, into an anterior portion termed the primite, and a posterior portion called the deutomerite. The single nucleus is almost invariably in the deutomerite.

The life history varies from a relatively simple and uncomplicated progression from sporozoite to sporozoite to a complex alternation of generations involving different hosts. The simpler histories are found in the Eugregarinida such as *Monocystis* species or in *Lankesteria ascidiæ* (Fig. 179). The latter is a parasite of the digestive tract of the ascidian *Ciona intestinalis* which becomes infected by eating contaminated food. The sporozoites are liberated from the sporoblasts and enter epithelial cells where they develop into gamonts. The adult forms are free in the lumen of the gut and are characterized by the possession of a peculiar pseudopodium-like knob which is regarded as a tactile organ. Two of these adults come together in "pseudoconjugation." A delicate membrane is formed and within this membrane each of the individuals forms a large number of gametes. From the great nucleus a smaller nucleus is formed and this divides repeatedly, its products passing to the periphery where small buds, each containing a nucleus are pinched off as gametes. A gamete from one individual meets and fuses with a gamete from the other. A fertilization membrane is formed which becomes the capsule of the sporoblast. The syn-

karyon divides three times and eight daughter nuclei are formed which become the nuclei of eight sporozoites. In each sporocyst, therefore, there is a possibility of as many zygotes and sporoblasts as there are gametes formed by one of the original gregarines. The parasites are passed out of the intestine with the feces and further development is inhibited until the sporoblasts are eaten by another host.

A more complex, but still simple life history involves a change of hosts. The genus *Porospora* appears to be represented by several species which pass their trophic stages in the digestive tract of crustacea and their sexual stages in mussels. *Porospora gigantea* grows to an enormous size (up to 16 mm.) in the lobster (*Homarus* sp.) where it apparently lives for a long period. Ultimately and either in association or individually, it becomes spherical and forms a cyst-like ball with a diameter of 3 to 4 mm. The ball then divides into many gametocytes each with a diameter of from 5 to 8 μ, and each gametocyte forms gametes which are arranged radially about a central residual body. The gametes are very small (3 μ long by 1 μ in diameter) and pass out with the feces into the water with which they enter the digestive tract of the mussel (*Mytilus edulis*) where they unite to form zygotes. Each zygote forms a single sporozoite which is liberated in the gut of the lobster.

The Schizogregarinida are more complicated through the introduction of an asexual reproductive phase in the life history leading to spread of the infection in the same host. Under the term "multiplicative reproduction" Doflein distinguishes this phase from the reproduction following fertilization which he calls "propogative reproduction." A relatively simple, but very interesting life cycle is described by Léger in the case of *Ophryocystis mesnili* found in the Malpighian tubules of the beetle *Tenebrio molitor* (Fig. 180). Here the asexual cycle is reduced to a process of equal division or multiple division whereby a number of gamonts are formed. These gamonts unite two by two in pseudoconjugation. The nucleus of each divides twice and one only of the resultant four nuclei becomes the nucleus of a gamete. The two gametes become freed in a brood chamber where they unite and in which the zygote gives rise to a single sporoblast with eight sporozoites.

In *Schizocystis sipunculi* and in *Eleutheroschizon dubosqui* the asexual cycle is represented by a process of multiple unequal division, the agametes being formed by a process of internal budding (Fig. 181).

In some cases, particularly in the cephalont gregarines, specialized sporoblast disseminating tubes known as sporoducts, are formed by the sporocysts. These are developed as ingrowths from the cortical protoplasm which in the ripe sporocyst and under the influence of moisture are evaginated as tubular processes through which

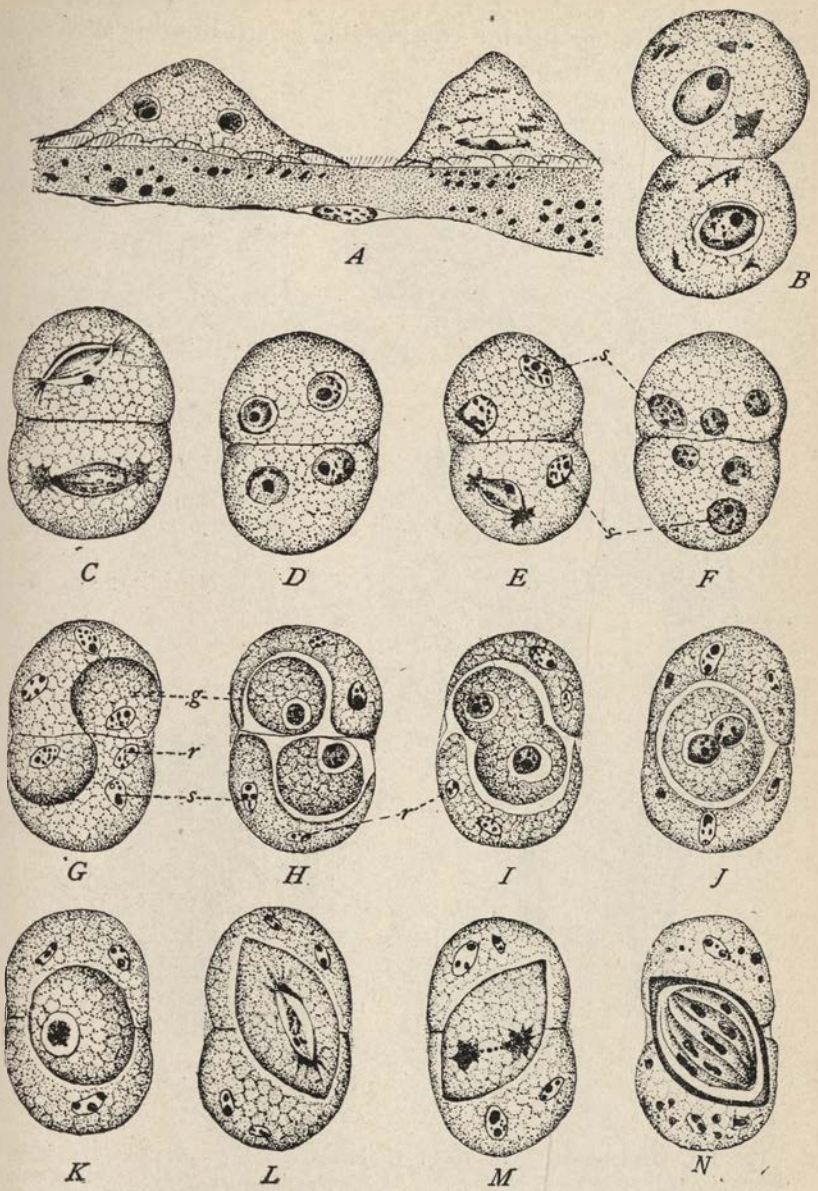

FIG. 180.—*Ophryocystis mesnili*, gamete formation and fertilization. *A*, Two individuals attached by processes to epithelial cells of a Malpighian tubule of *Tenebrio mollitor; B*, union of gamonts in pseudoconjugation; *C, D, E*, divisions (probably meiotic) of nuclei of the two gamonts; *G* to *K*, formation of two gametes and their union in fertilization; *L* to *N*, metagamic divisions resulting in eight sporozoites in the single sporoblast. (After Léger.)

the sporoblasts are emitted (Fig. 182). In *Gregarina ovata* they are quite short but reach a considerable length in other species of Gregarina and in Clepsidrina.

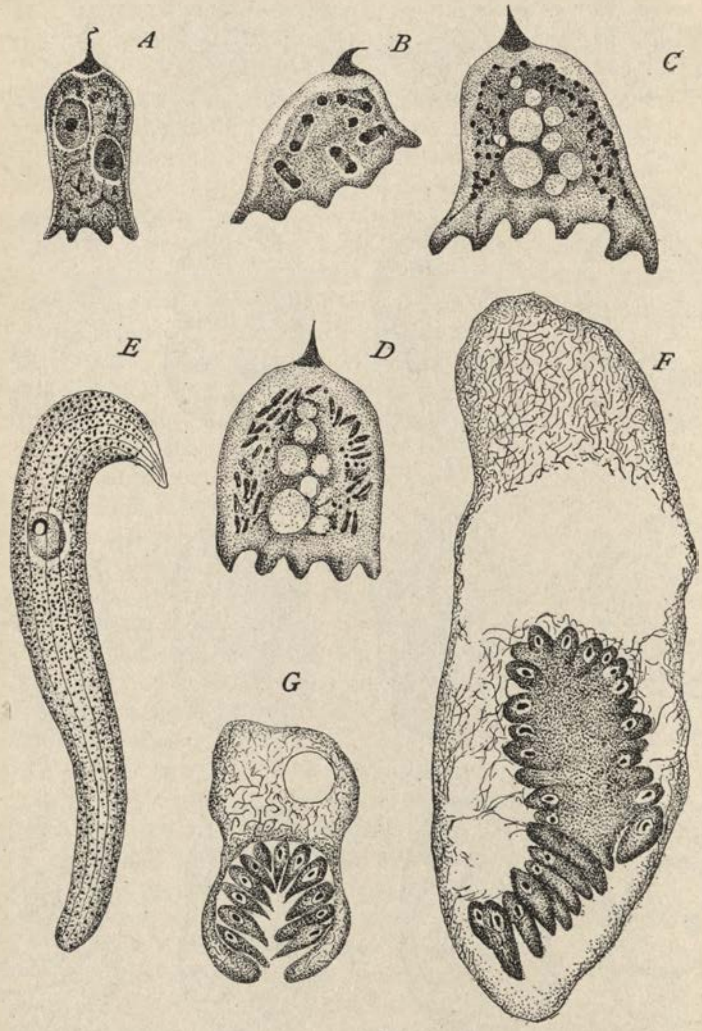

FIG. 181.—Endogenous budding in Gregarinida. *A* to *D*, *Eleutheroschizon dubosqui* and formation of endogenous agametes. *E* to *G*, *Schizocystis sipunculi* and similar formation of agametes. (*A* to *D*, after Brasil, *E* to *G*, after Dogiel.)

Gamete dimorphism is highly variable in different species of gregarines. Isogametes are produced by some species of *Monocystis*, anisogametes by others although here the differences are slight.

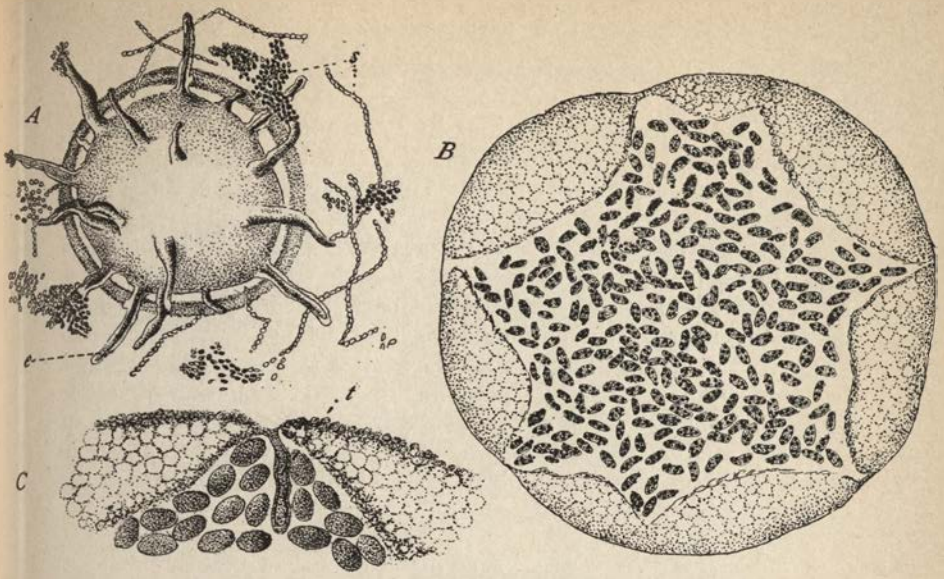

FIG. 182.—*Gregarina cuneata*. *A*, surface view of sporocyst with ripe sporoblasts issuing from sporoducts (*e*). *B*, *C*, sections of sporocyst with ripening spores and developing sporoducts (*t*). (From Calkins after Kuschakewitsch.)

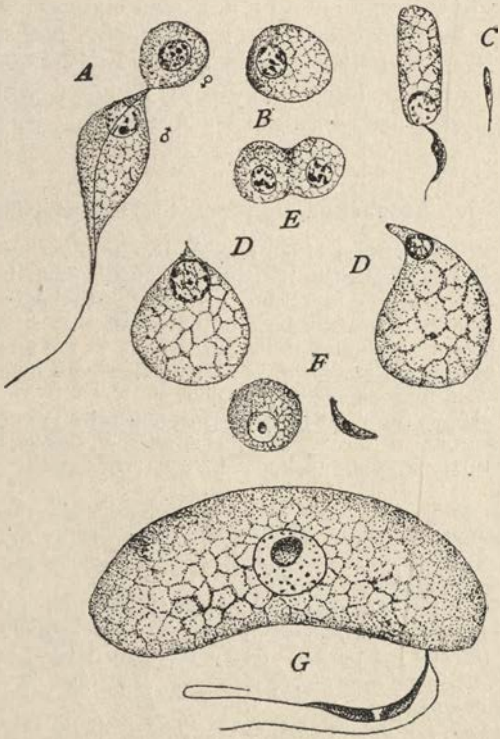

FIG. 183.—Gametes of Gregarines and Coccidia. *A*, male and female gametes of *Stylorhynchus longicollis*; *B*, *Monocystis* sp.; *C*, spermatozoid of *Echinomera hispida*, to the left the two gametes of *Pterocephalus nobilis*; *D*, gametes of *Urospora lagidis*; *E*, of *Gregarina ovata*; *F*, of *Schaudinella henleæ*; and *G*, *of Eimeria schubergi*. (From Shellack after Léger, Cuénot, Brasil, Schnitzler and Schaudinn.)

Well-marked anisogamy is found in *Pterocephalis nobilis* (Duboscq and Léger) and in *Schaudinnella henleæ* (Nusbaum), but in general, differences in gametes are much less pronounced than in the Coccidiomorpha (Fig. 183).

The sporoblasts in different species vary widely in form and in sculpturing. The capsule is usually double consisting of an inner (endospore) and an outer (exospore) capsule, the latter sometimes provided with short spines (*Acanthospora*) or long filaments (*Ceratospora*). The typical number of sporozoites in a sporoblast is eight but this is not invariable. They are liberated by action of gastric juices and emerge through preformed openings or by separation of the two valves of the sporoblast. They creep out of the endospore and make their way to epithelial cells within which the first stages of their development occur.

Order 1. **Eugregarinida,** Doflein Emend.

The great majority of known gregarines belong to this Order, the agamous individuals living for long periods in the host before uniting in couples to form isogamous or anisogamous gametes. Division or asexual reproduction of any kind is unknown. Only exceptionally are more, or less, than eight sporozoites formed in each sporoblast. They are monocystid (single chambered) or polycystid in structure the former grouped in the Sub-order Acephalina, the latter in the Cephalina.

Sub-order 1. **Acephalina,** Koelliker **(Monocystidea,** Stein**).**

1. Genus *Monocystis*, Stein (1848). The trophozoites are often highly contractile owing to the peristalsis brought about by the contractions of ectoplasmic myonemes. Spores boat-shaped and octozoic. Many species from worms and entomostraca, a typical species, *M. agilis* may be found almost invariably in the seminal reservoirs of the common earthworm, and excellent stages in sporulation and fertilization may be easily obtained.
2. Genus *Zygocystis*, Stein (1848). The trophozoites are usually found in pairs or groups of three. Typical species, *Z. cometa*, Stein, found in the seminal vesicles and body cavity of the earthworm *Lumbricus agricola*.
3. Genus *Zygosoma*, Labbé (1899). The trophozoite has typical and characteristic finger-like processes and is usually found in couples. Sporulation unknown. Typical species, *Z. gibbosum*, Greeff (1880), in the gut of *Echiurus pallassii*.
4. Genus *Pterospora*, Racovitza and Labbé (1896). The pyriform trophozoites are always associated in couples. The spores have dissimilar poles and the epispore is drawn out into lateral processes. One species, *P. maldaneorum*, R. and L., from the cœlomic cavity of maldanid worms.
5. Genus *Cystobia*, Mingazzini (1891). The trophozoites are large and irregular in form and usually have two nuclei due to the early fusion of two individuals. The spores are heteropolar, and the epispore is

drawn out into chimney-like projections at one pole. One species, *C. holothuriæ*, A. Sch., from the blood vessels and body cavity of holothurians.

6. Genus *Lithocystis*, Giard (1876). The trophozoite is characterized by an endoplasm filled with crystals of calcium oxalate. The epispore has long processes. A single species from the cœlomic cavities of various echinids.
7. Genus *Ceratospora*, Lèger (1892). The trophozoites fuse by their truncated ends and give rise to spores without encysting. The spores are characterized by long spinous processes. A single species, *C. mirabilis*, Lèger, from the body cavity of Glycera.
8. Genus *Urospora*, A. Schn. (1875). The spores are characterized by the presence of a long caudal filament at one pole. Several species from the body cavities of oligochetes, nemertines, sipunculids, and other marine invertebrates.
9. Genus *Gonospora*, A. Schn. (1875). The trophozoites are quite variable in form and give rise to heteropolar spores bearing from one to several tooth-like processes at one pole, and rounded at the other. Four species from the body cavities of polychaetous worms.
10. Genus *Syncystis*, A. Schn. (1886). The spores are ovoid or boat-shaped, with spines or processes at each extremity. One species, *S. mirabilis*, A. Schn., from fat body and cœlom of species of Nepa.
11. Genus *Diplocystis*, Kunstler (1887). The trophozoites fuse precociously to form spherical masses of gregarines in the body cavity of crickets and cock-roaches. The spores are either spherical or oblong.
12. Genus *Lankesteria*, Mingazzini (1891). The spores are more or less flattened or spatulate, oval in outline, and octozoic. Type species, *L. ascidiæ*, Lank, from the gut of *Ciona intestinalis*.
13. Genus *Callyntrochlamys*, Frenzel (1885). The trophozoites have a central constriction but no septum dividing the body into protomerite and deutomerite; they are covered by a fur-like fringe of processes resembling cilia. The spores are unknown. Type species, *C. phronimæ*, Frenz., from the gut of *Phronima sedentaria*.
14. Genus *Ancora*, Labbé (1899). The trophozoite has a peculiar anchor-like form by reason of two lateral bulgings of the body. Spores unknown. Species, *S. sagittata*, Leuck. from the gut of *Capitella capitata*.

Sub-order 2. **Cephalina,** Delage **(Polycystidea,** Stein**).**

Eugregarines possessing an epimerite at some stage of the life history either in the adult phase or during the temporary young phases. The body is usually divided by a septum into protomerite and deutomerite and the gamonts are frequently associated in couples arranged tandem each couple consisting of an anterior primite and a posterior satellite. Parasites confined mainly to the digestive tracts of various arthropods. The classification follows Watson (1916) with only minor changes.

Family 1. **Gregarinidæ,** Labbé (1899). Individual solitary or associated (with satellites), in latter case with septum. Epimerites simple and symmetrical. Cysts with or without sporoducts.

1. Genus *Gregarina*, Dufour (1828). Biassociative. Epimerite small, cylindrical or globular; cysts open by sporoducts.
2. Genus *Hirmocystis*, Labbé (1899). Individuals associated in groups of two to twelve. Epimerite a small papilla; cysts open by simple rupture; sporoblasts ovoidal.

3. Genus *Hyalospora*, Schneider (1875). Biassociative; epimerite a small knob; cysts open by rupture; endoplasm yellow-orange; sporoblasts ellipsoidal. Gut of Petrobius sp.
4. Genus *Cnemidospora*, Schn. (1882). Solitary; protomerite globular anterior half gray, posterior half yellow-green; cysts open by simple rupture; spores ellipsoidal. Gut of Glomeris.
5. Genus *Euspora*, Schn. (1875). Biassociative; no sporoducts; sporoblast prismatic. Gut of *Rhizotrogus estivus*.
6. Genus *Sphærocystis*, Léger (1892). Solitary; protomerite temporary, body spherical; cysts without sporoducts, sporoblasts ovoidal.
7. Genus *Gamocystis*, Léger (1892) Schn. (1875). Associative; protomerite only in young stages, cyst with sporoducts, sporoblasts cylindrical and elongate. Cock-roach and other insects.
8. Genus *Frenzelina*, Léger and Dub. (1907). Biassociative, cysts without sporoducts, sporoblasts ovoidal with a dark equatorial line, epimerite unknown.
9. Genus *Uradiophora*, Mercier (1912). Bi- or triassociative, epimerite a simple style forked at end, cysts without sporoducts, sporoblasts dolioform.
10. Genus *Leidyana*, Watson (1915). Solitary; epimerite a globular knob; cyst with sporoducts; sporoblasts dolioform.

Family 2. **Stenophoridæ**, Léger and Duboscq (1904). Individuals solitary; epimerite absent or a mere knob; cysts without sporoducts; sporoblasts oval with broad exospore and with equatorial line, sporoblasts not extruded in chains.

11. Genus *Stenophora*, Labbé (1899). Gamonts large with rudimentary epimerite, sporoblasts with dark sutural line, intestine of millipedes.
12. Genus *Oöcephalus*, Schn. (1886). Epimerite a spherical knob on a short conical neck.

Family 3. **Didymophyidæ**, Léger (1892). In associations of two or three, satellites without septa.

13. Genus *Didymophyes*, Stein (1848). The epimerite has the form of a small pointed protuberance, sporocyst without sporoducts, sporoblasts ellipsoidal, gut of species of Aphodius.

Family 4. **Dactylophoridæ**, Léger (1892). Individuals solitary; epimerites asymmetrical and irregular with digitiform processes; sporocysts open by simple rupture or by swelling of a residual mass of plasm termed a "pseudocyst;" sporoblasts elongate, cylindrical or ellipsoidal.

14. Genus *Dactylophorus*, Balbiani (1889). The protomerite is dilated excentrally and bears epimerite with digitiform processes, individuals solitary; sporocysts open by swelling of lateral pseudocyst, sporoblasts cylindrical and emitted in oblique chains, gut of *Cryptops hortensis*.
15. Genus *Nina*, Grebnecki (1873). Protomerite formed of two long and narrow horizontal arms turned up at ends, and said to bear a small nucleus (?), the attaching surface bears teeth from which filaments arise, sporoblasts emitted in oblique chains, gut of myriapods.
15. Genus *Trichorhynchus*, Schn. (1882). Protomerite truncated, epimerite elongated and conical, sporocysts open by lateral pseudocysts, sporoblasts not in chains, gut of Scutigera.
16. Genus *Echinomera* Labbé (1899). Epimerite an excentric cone with eight or more short digitiform processes from the sides, sporocysts open by simple rupture, sporoblasts cylindrical and in chains, gut of *Lithobius fortificatus*.

17. Genus *Rhopalonia*, Léger (1893). Solitary; epimerite a subspherical cushion with ten or more short finger-form processes, pseudocyst present, sporoblasts cylindrical, gut of different myriapods.
18. Genus *Acutispora*, Crawley (1903). Sporocysts open by pseudocysts, sporoblasts biconical with endospore plug at each end, epimerite not seen, gut of *Lithobius fortificatus*.
20. Genus *Metamera*, Duke (1910). Epimerite conical with excentric apex surrounded by numerous branched processes, sporocysts open by simple rupture, sporoblasts biconical, gut of leeches.

Family 5. **Actinocephalidæ**, Léger (1892). Individuals always solitary with variable epimerites; sporocysts open by simple rupture; sporoblasts biconical, cylindrical, or navicular.

21. Genus *Sciadophora*, Labbé (1899). The epimerite is large and flattened, protomerite umbrella-shape, with radiating ridges, sporoblasts biconical, gut of Phalangium sp.
22. Genus *Anthorhynchus*, Labbé (1899). Epimerite in the form of a large grooved knob or button, sporoblasts ovoidal, pointed, gut of *Phalangium opilio*.
23. Genus *Pileocephalus*, Schn. (1875). Epimerite lance-like or simply conical, sporoblasts biconical, different species in intestines of *Necrobia ruficollis*, *Blabera claraziana*, and *Mystacides* of the Coleoptera, Orthoptera and Neuroptera respectively.
24. Genus *Amphoroides*, Labbé (1899). Protomerite short and cup-like, epimerite a globular papilla, sporoblasts curved, gut of myriapods.
25. Genus *Discorhynchus*, Labbé (1899). Protomerite larger than deutomerite which is cylindrical and truncated posteriorly, epimerite large and globular with a thin collar around the base, cysts spherical; sporoblasts biconical and slightly bent; gut of neuropteron, Sericostoma sp.
26. Genus *Stictospora*, Léger (1893). Epimerite with globular head depressed ventrally and covered with ribs which project posteriorly as spikes; sporoblasts biconical and slightly curved; intestines of species of Melolontha and Rhizotrogus larvæ.
27. Genus *Schneideria*, Léger (1892). Gamonts without protomerite; epimerite a thick plate bordered by rib-like thickenings; sporoblasts biconical; intestines of *Bibio marci* and *Sciara nitidicollis* larvæ, Diptera.
28. Genus *Asterophora*, Léger (1892). Epimerite a circular disc with ribs surrounding a prominent central style; sporoblasts cylindrical with conical ends, intestines of insect larvæ.
29. Genus *Bothriopsis*, Schn. (1875). Epimerite an ovoidal structure with six or more long slender filaments; individuals motile; transverse septum convex anteriorly; sporoblasts biconical, gut of *Hydaticus cinereus*.
30. Genus *Coleorhynchus*, Labbé (1899). Protomerite a circular shallow disc depressed sucker-like in center, the septum projects into the protomerite, sporoblasts biconical or navicular, gut of *Nepa cinerea*.
31. Genus *Legeria*, Labbé (1899). Protomerite enlarged and club-like with invading septum as above, sporoblasts sub-navicular, gut of Colymbetes sp.
32. Genus *Phialoides*, Labbé (1899). Complex stalked epimerite consisting of a discoid retractile cap surrounded by a circular ridge with a collar-like membrane and with ridges ending in triangular teeth, sporoblasts biconical, gut of Hydrophilus larvæ.

33. Genus *Geniorhynchus*, Schn. (1875). The epimerite is discoid and borne on a long neck and bears a tuft of short bristles; sporoblasts subnavicular; gut of nymphs of Libellulidæ.
34. Genus *Actinocephalus*, Stein (1848). Epimerite small, sessile or borne on a short neck, with from eight to ten sharp spines or simple bifurcate processes; sporoblasts biconical; several species from the intestines of beetles.
35. Genus *Pyxinia*, Hammerschmidt (1838).—The epimerite is a flat crenulate disc from the center of which rises a short or long style; sporoblasts biconical; many species in digestive tracts of Coleoptera.
36. Genus *Beloides*, Labbé (1899). Epimerite in the form of a disc or knob bearing about ten teeth in addition to a long spike; sporoblasts navicular or oval; in digestive tracts of species of Dermestes.
37. Genus *Stylocystis*, Léger (1899). Epimerite in the form of a long spine which is usually curved; sporoblasts biconical; larva of Tanypus sp.
38. Genus *Tæniocystis*, Léger (1906). Epimerite a short sphere set with six or eight recurved hooks; deutomerite divided by transverse septa into numerous transverse segments; sporoblasts biconical; gut of the neuropteron Sericostoma.
39. Genus *Hoplorhynchus*, Carus (1863). Epimerite a flat button with eight to ten finger-form processes carried on a long collar; sporoblasts biconical; digestive tract of myriapods.
40 Genus *Amphorocephalus*, Ellis (1913). Epimerite dilated in the middle and terminates in a concave disc with a fluted periphery; the protomerite is constricted across the middle; sporoblasts unknown; gut of *Scolopendra heros*.
41. Genus *Steinina*, Léger and Duboscq (1914). Epimerite a short mobile finger-form process which may change into a flattened button; sporoblasts biconical; several species in different species of Coleoptera.

Family 6. **Acanthosporidæ,** Léger (1892). Gamonts always solitary; epimerites simple or with appendages; sporocysts open by simple rupture; sporoblasts ornamented with bristles at the poles or at the equator.

42. Genus *Corycella*, Léger (1892). Protomerite spherical and somewhat dilated; epimerite a knob with a crown of eight large recurved hooks; sporoblasts biconical with four spines at each pole; digestive tract of *Gyrinus natator*.
43. Genus *Acanthospora*, Léger (1892). Epimerite a simple conical knob; sporoblast biconical or oval with a girdle of equatorial spines and a group of four spines at each pole; species in the gut of Omoplus sp. and Cistelides sp. larvæ.
44. Genus *Ancyrophora*, Léger (1892). Deutomerite pointed; epimerite a knob with appendages in the form of recurved hooks; sporoblasts biconical with polar tufts and six equatorial bristles; two species from various Coleoptera.
45. Genus *Cometoides*, Labbé (1899). Epimerite a spherical knob flattened centrally and bearing a circlet of flexible filaments; sporoblast with a tuft of bristles at each pole and two circlets of bristles about the equator; two species from the Coleoptera Hydrous sp. and Hydrobius sp.

Family 7. **Stylocephalidæ,** Ellis (1912). Gamonts solitary; epimerites varied; sporoblasts irregular in shape, brown or black, and in chains.

46. Genus *Stylocephalus*, Ellis (1912). Epimerite a dilated knob at the end of a long and slender neck; sporocyst marked by small papillæ and indentations; sporoblasts hat-shape; several species in Crustacea, Phalangidæ, and Coleoptera.

47. Genus *Sphærocystis*, Labbé (1899). Epimerite a small sphere or ellipsoid at the end of a long slender neck; gut of *Cyphon pallidulus*.
48. Genus *Lophocephalus*, Labbé (1899). Epimerite large, sessile flat and cup-like disc with crenulate margin and numerous upright digitiform processes; sporoblasts black and hat-shape; gut of *Helops striatus*.
49. Genus *Cystocephalus* Schn. (1886). Epimerite a large lance-shape papilla set on a short stout cylindrical neck; sporoblasts of irregular shape; gut of Pimelia sp.

Family 8. **Menosporidæ**, Léger (1892). Gamonts solitary; epimerite a large cup bordered with hooks and placed on a long slender neck; sporocysts open by simple rupture; sporoblasts crescentic, smooth.

50. Genus *Menospora*, Léger (1892). Characters of the family; gut of Agrion sp.

Order 2. **Schizogregarinida**, Léger (1892).

The Schizogregarinida are parasites of the digestive tract and appended organs of arthropods, annelids and tunicates. They differ from the Eugregarinida in having an asexual or multiplicative cycle, the sporozoite growing into an agamont either as an intracellular or an extracellular parasite. Asexual reproduction occurs by division, internal budding or by multiple division. The life history, gamete formation and metagamic divisions of the zygote vary widely and no characteristic difference marks the sporoblasts from those of the Eugregarinida. Change of hosts is safely established for only one type—the Porosporidæ. According to the presence of one or of more than one sporoblast in a sporocyst Léger and Duboscq divide the group into subdivisions, the Monospora and the Polyspora. Systematically this is preferable to the subdivisions into Entoschiza and Ectoschiza as suggested by Fantham on the basis of the mode of parasitism of the young stages. Owing to absence of information in connection with the life history of the majority of forms it seems wiser at present to follow Doflein in cutting out further subdivisions entirely except for the families as follows.

Family 1. **Ophryocystidæ**, Léger and Duboscq (1900). These are the best known of all the schizogregarines, the full life history having been worked out by Léger (1907). They are extracellular parasites of the Malpighian tubules of beetles with asexual reproduction by simple or multiple division. The single sporoblast (see above p. 424) is characteristic but its structure differs in the many different species. The sporocyst membrane is single or multiple (Fig. 184) and the number of sporozoites is eight.

1. Genus *Ophryocystis*, A. Schneider. With the characters of the family.

Family 2. **Schizocystidæ**, Léger and Duboscq. Cylindrical or elongated with a differentiated anterior end. Agamogony by multiple division, extracellular. Pseudoconjugation, gamete formation and sporoblast formation with eight sporozoites, as in Eugregarinida.

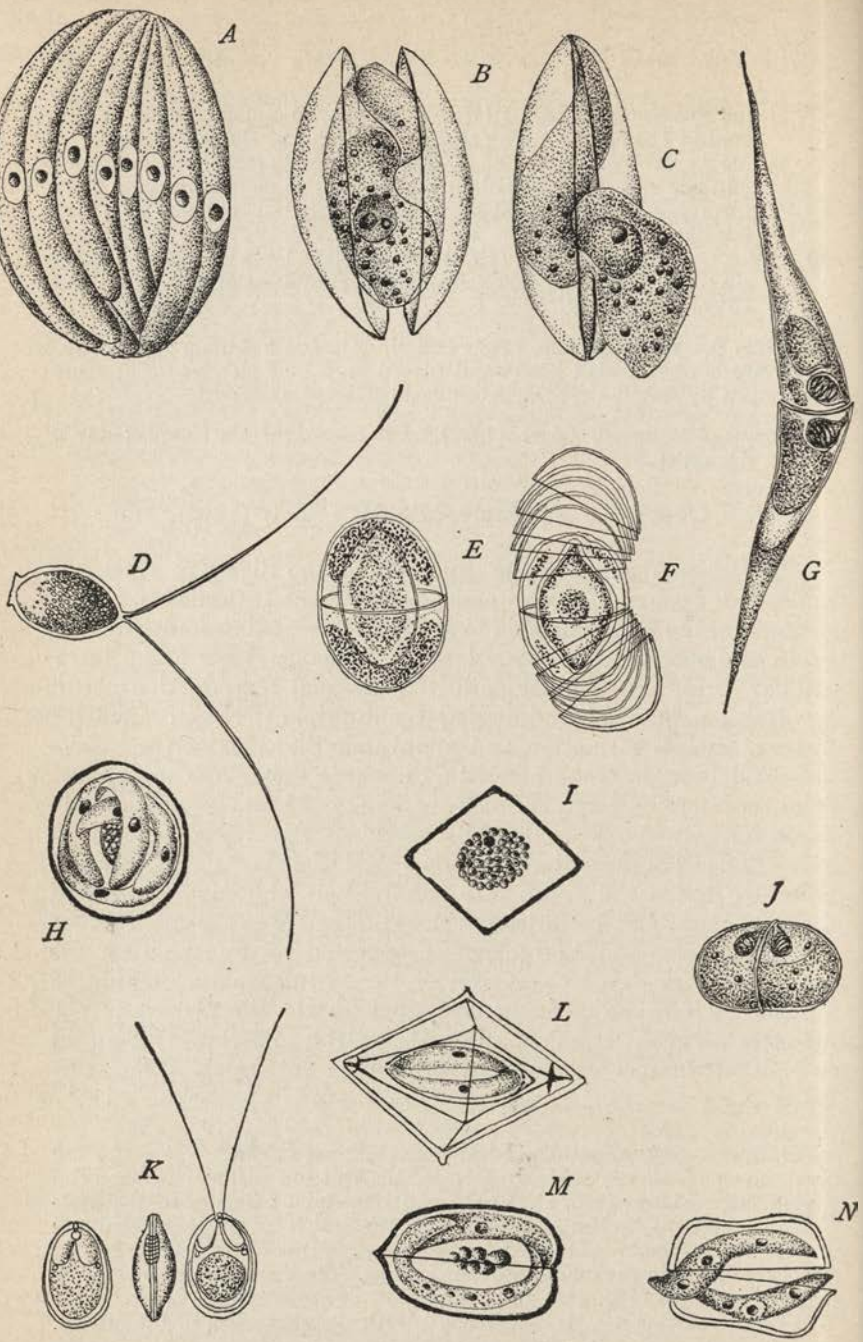

FIG. 184.—Reproductive bodies in Sporozoa. *A*, agametes of *Barrouxia ornata; B, C*, sporoblasts of same with exit of sporozoites; *D*, tailed sporoblast of *Urospora lagidis; E. F*, sporoblast of *Ophryocystis mesnili* with single and multiple spore cases; *G*, spore of *Ceratomyxa* sp.; *H*, coccidian sporoblast with four sporozoites; *J*, spore of *Leptotheca agilis; K*, type of *Myxobolus* spore; *L*, sporoblast of *Crystallospora crystalloides; M, N*, coccidian sporoblast with two sporozoites. (After Schneider, Wasielewsky, Thélohan, Léger and Brasil.)

2. Genus *Schizocystis*, Léger (1909). With the characters of the family; parasites of marine annelids and sipunculids.
3. Genus *Eleutheroschizon*, Brasil (1906). Agamogamy by internal budding. Parasite of scoloplos.

Family 3. **Seleniidæ**, Brasil (1905). Highly motile worm-like forms parasitic in marine annelids and in Gephyrea. An epimerite-like attaching organ and myonemes running the entire length of the body are present. The sporozoites develop as intracellular parasites into agamonts which reproduce by multiple division. The gamonts unite in pseudoconjugation and form isogametes as in Eugregarinida; the sporoblasts are spherical, provided with spines and give rise to four sporozoites.

4. Genus *Selenidium*, Giard. With the characters of the family.
5. Genus *Selenococcidium*, Léger and Duboscq (1910). Similar to *Selenidium* in form and agamous reproduction but very much like the Coccidia in gamete formation and absence of pseudoconjugation. Parasite of the lobster; sporoblast formation unknown.

Family 4. **Merogregarinidæ**, Porter (1909). Parasites of the ascidian Amourœcium (?); sporozoites and agamonts intracellular; sporoblasts with eight sporozoites.

6. Genus *Merogregarina*, Porter. With characters of the Family.

Family 5. **Porosporidæ**, Léger. These are large forms (up to 16 mm.) found in the digestive tract of Crustacea. Full development is unknown; in one species—*Porospora legeri* Beauchamp—two individuals become encysted together and undergo what is reported to be agamogony, or the formation of an immense number of so-called "gymnospores." In *P. gigantea* the individuals undergo such a process of reproduction singly, the "gymnospores" developing as gamonts in the mussel *Mytilus edulis*. It is quite possible that such "gymnospores" are gametes in which case the Porosporidæ should be included with the Eugregarinida. Change of host appears to be obligatory in Crustacea and lamellibranch mollusks.

7. Genus *Porospora*, A. Schn. (1875). Several species with characters as above.

Family 6. **Spirocystidæ**, Léger and Duboscq. Forms infesting practically all of the organs of *Lumbriculus variegatus* Mull. Agamogony and gamogony occur in the same host, sporoblasts with one sporozoite, agamonts spirally wound or crescentic.
8. Genus *Spirocystis nidula*, Léger and Duboscq; characters as above.

Family 7. **Caulleryellidæ**, Keilin. Intestinal parasite of the larva of the dipteron *Aphiochæta rufipes*. The agamont gives rise to sixteen agametes, gamonts come together in pseudoconjugation each forming eight gametes, sporoblasts elongate, oval, each with eight sporozoites.

9. Genus *Caulleryella*, Keilin; characters as above.

Sub-class II. **COCCIDIOMORPHA**, Doflein.

While the Gregarinida are practically limited to invertebrate hosts and are typically lumen-dwelling parasites, the Coccidiomorpha are widely distributed in all groups of animals and are typically intracellular parasites in all stages of growth and repro-

duction. Change of hosts with alternation of generations while by no means universal, is more common than in the Gregarinida. Agamogony is characteristic of all types and leads to multiple infection with frequently lethal results to the host due to the destruction of multitudes of epithelial or blood cells, to thrombus formation, or to the liberation of toxins. The life cycle varies from relative simplicity to great complexity; gamonts become differentiated into gametocytes which may be recognized as male and female; gametes are anisogamous with rare exceptions; zygotes give rise to sporoblasts which may (Coccidia) or may not (Hæmosporidia) be protected by resistant membranes. The presence or absence of sporoblast capsules is of primary importance in the mode of life and the life history, and affords an excellent basis for the natural classification of the group into two Orders, Coccidia and Hæmosporidia, epithelium and blood-dwelling parasites respectively.

Order 1. **Coccidia,** Leuckart, Em.

Typically epithelial-cell dwelling parasites with encapsulated sporoblasts. Exceptions to both generalizations, however, are known; thus *Cryptosporidium muris*, Tyzzer; *Eimeria mitraria*, Lav. and Mesn., and *Orcheiobius herpobdellæ*, Kunze, are lumen-dwelling coccidia, while in *Dobellia* and in *Legerella* sporoblast capsules are absent. Hemogregarines are not epithelial cell parasites but live in blood cells both leukocytes and red cells. In other respects the group is fairly homogenous and the life history as outlined on p. 418 is typical for practically all species. Variations in details as for example time (before or after fertilization) of formation of the sporocyst capsule, number of sporoblasts formed by the zygote, the presence or absence of a residual body in sporocyst or sporoblast, and the number of sporozoites in a sporoblast are useful in distinguishing genera and species.

Anisogamous gametes are typical throughout the group. In some cases they approach the gregarine type with pseudoconjugation, although cyst membranes are not formed. Thus in Adeleidæ, some types, for example *Adelea* and *Dobellia*, are represented by gametocytes which come together in pseudoconjugation; one, the microgametocyte undergoes nuclear division until usually four (more in *Dobellia*) microgamete nuclei are formed. One of these nuclei penetrates the macrogamete and fertilizes it. In other cases fertilization is brought about by wandering flagellated microgametes.

Cellular differentiations are much less numerous than in the gregarines, particularly is this true of the cortex. They are motionless forms without myonemes or other motile organs save flagella of the microgametes, and cellular processes are generally absent. The endoplasm is usually well stored with products of metabolism some

of which are so characteristic that they have received the name of coccidin. They are all osmotic in nutrition, and infection is always, so far as known, by the contaminative method through the digestive tract. The sporozoite penetrates an epithelial or other definitive cell, grows at the expense of the cell which it ultimately destroys, and forms agametes while still intracellular. *Cyclospora karyolytica*, Schaud. of the ground mole enters the nucleus of the intestinal epithelial cell and as a karyozoic parasite completes its life history.

We follow Doflein in dividing the Coccidia into two families the Adeleidæ and the Eimeriidæ, each further divided into sub-families as follows:

Family 1. **Adeleidæ,** Léger. Forms with a small number, usually four, of non-flagellated gametes derived from a microgametocyte, macro- and microgametocytes sexually differentiated and united in more or less youthful stages in pseudoconjugation. Four sub-families are recognized, three of them characterized by Léger but the grouping should be regarded as tentative until further knowledge of the different genera is forthcoming.

Sub-family 1. **Legerellinæ,** Léger. The single genus included here is said to be characterized by sexually differentiated agametes. Sporozoites are formed directly by the zygote without sporoblasts, zygote with two or three cyst membranes.

1. Genus *Legerella*, Mesnil. With four microgametes; Malpighian tubules of species of Glomeris.

Sub-family 2. **Adeleinæ,** Léger. Sporozoites two or four in number in each sporoblast, sporoblasts oval and discoid four or more in number in each sporocyst.

2. Genus *Adelea*, A. Schn. Many sporoblasts formed in each cyst; each sporoblast with two sporozoites arranged tête a tête; gut of *Lithobius fortificatus* and of insects.
3. Genus *Adelina*, A. Schn. With thick-walled spherical sporoblasts formed in a thick walled cyst; different tracheates.
4. Genus *Chagasia*, Léger. A possibly allied form with sporoblasts each with four sporozoites; from the Brazilian hemipteron *Dysdercus ruficollis* L.
5. Genus *Hyaloklossia*, Labbé (1899). Numerous sporoblasts with two or four sporozoites.
6. Genus *Minchinia*, Labbé (1899). Many sporoblasts each with two sporozoites and with two long threads; liver of Chiton and Patella species.
7. Genus *Klossia*, A. Schn. (1875). Sporocyst with a large number (up to 160) of globular sporoblasts each with four sporozoites; kidney parasites of land snails (species of Helix and Succinia).
8. Genus *Orcheobius*, Kunze (1907). Macrogamete very large and worm-like; microgametocyte much smaller forming four microgametes, sporoblasts globular, twenty-five to thirty in number and each with four sporozoites, testis of *Herpobdella atomaria* (*Nephelis vulgaris*).

Sub-family 3. **Hæmogregarinæ,** Léger (in part) Fig. 177. The hemogregarines are Adeleidæ parasitic in the blood cells of vertebrates in which the asexual phase occurs, the sexual phase is carried on for the most part

in the digestive tract of some blood-drawing invertebrate. The red cells of fish, amphibia, reptiles, birds and mammals may be infested and in some cases the white cells; blood-sucking leeches, ticks, lice and fleas supply the needed environment for the sexual generation. In form and in movement as well as in pseudoconjugation they recall the gregarines.

9. Genus *Hæmogregarina*, Danilewsky (1885). Life history complex and for the larger number of species incomplete. The best known species is *H. stepanowi* of the turtle the life history of which, worked out by Reichenow, is very complex. The agametes are formed as a result of multiple division and are about twenty-four in number in the red blood cells of the turtle, ultimately the agamonts instead of forming twenty-four products form only four which develop into gametocytes. These conjugate in the gut of the leech as in *Adelea*, the microgametocyte forming four microgametes, the zygote gives rise to eight sporozoites, hosts, turtles and leeches (*Placobdella catenigera* for *H. stepanowi*).
10. Genus *Karyolysus* Labbé (1894). Blood parasites of reptiles (snakes, turtles and lizards), *K. lacertarum*, Danil. of the lizard best known, here individuals are dimorphic, one type forming larger agametes the other, smaller, the larger agametes serve for asexual reproduction, the smaller, form gametocytes (Reichenow) which form gametes in *Liponyssus saurarum;* two individuals of similar size come together in pseudoconjugation and form a delicate cyst wall, the macrogamete grows much larger than the microgametocyte which forms two microgametes, the zygote forms twenty to thirty sporozoites, infection of new host is brought about by feeding on infected mites.
11. Genus *Hepatozoön*, Miller (1908). Hæmogregarine of the rat with sexual cycle in the mite *Lelaps echidninus;* rats infected by eating infected mites, sporozoites liberated in rat's intestine penetrate the gut wall and enter the blood stream where they are carried to the liver, agamonts develop in liver cells where agamogony occurs with the formation of from twelve to twenty agametes, some penetrate leukocytes in the blood and develop into gametocytes. These taken into the mite unite in pairs in the gut, the macrogamete becomes very large and partly encircles the microgametocyte which becomes a single microgamete; fusion is complete and the zygote penetrates the intestinal wall of the mite and encysts in the body tissues; it ultimately forms from fifty to a hundred sporoblasts with capsules, and each sporoblast forms about sixteen sporozoites. A similar history is described by Christophers (1906) and by Wenyon (1911) for *H. canis* of the dog with transmission and sexual cycle in *Rhipicephalus sanguineus*, and several other species are known from rodents other than the rat.
12. Genus *Lankesterella*, Labbé. Small worm-like forms in the red blood cells of the frog and urodeles the full life history of which is unknown although many hypotheses have been given.

Sub-family 4. **Dobellinæ**, Ikeda (1914). The one genus and species included in this group is similar to other Adeleidæ in forming pairs in pseudoconjugation but differs from others in having a small microgametocyte which forms many microgametes. According to Ikeda the agametes are sexually differentiated and have different relations with the host cells, those destined to form macrogametes penetrate the nucleus of the host cell and later become cytoplasmic, those destined to form microgametocytes develop on the periphery of the host cell. The zygote forms about a hundred sporozoites without capsules.

13. Genus *Dobellia*, Ikeda (1914). Parasite of the gut of Sipunculidæ (*Petalostoma minutum*).

Family 2. **Eimeriidæ**, Léger. The forms included here, generally known as the Coccidia, differ from the Adeleidæ in having flagellated microgametes and typical fertilization without pseudoconjugation of gametocytes. Sporoblasts encapsulated for the most part. We follow Doflein in grouping the many genera in ten sub-families as follows.

Sub-family 1. **Cryptosporinæ**, Léger. The single genus and species of this group forms one of the interesting exceptions to the usual intracellular habitat of Coccidia. Like gregarines, it is a lumen-dwelling parasite of the peptic glands of the mouse. Sporoblasts are either absent or the sporocyst may be interpreted as forming one sporoblast with its capsule closely applied to the sporocyst membrane, four sporozoites. The sporozoites may develop in the same host thus leading to autoinfection.

1. Genus *Cryptosporidium*, Tyzzer (1908). Small forms in the stomach glands of the mouse.

Sub-family 2. **Cyclosporinæ**, A. Schn. The single genus included here is characterized by the small number of reproductive bodies formed as a result of fertilization. The agametes are said to be sexually differentiated (Schaudinn), the zygote forms two sporoblasts each with two sporozoites.

2. Genus *Cyclospora*, A. Schn. The best known species is *C. caryolytica*, Schn., a nuclear parasite of the intestinal epithelium of the ground mole.

Sub-family 3. **Caryosporinæ**, Léger. The zygote forms only one sporoblast with eight sporozoites.

3. Genus *Caryospora*, Léger (1904). Sporocyst a thick yellow membrane with a knot-like thickening at one point; sporoblast slightly pointed at one end; intestinal epithelium of the asp, *Viperia aspis* L.
4. Genus *Pfeifferinella*, Wasielewski (1904). The sporoblast has eight sporozoites and a conspicuous granular residual mass; parasitic in snails Planorbis and Succinia.

Sub-family 4. **Isosporinæ**, Léger (1911). The zygote forms two sporoblasts each of which has four sporozoites in capsules.

5. Genus *Isospora*, A. Schn. (1881). Many different species have been described some of which may turn out to be new genera when the full life history is known; parasites in widely different animal groups, slugs (*I. rara*, A. Schn.), cats and dogs and possibly man (*I. bigemina*, Stiles); birds (*I. lacazei*, Labbé), and lizards (*I. mesnili*, Sergent).

Sub-family 5. **Eimerinæ**, Léger (1911). The zygote forms four sporoblasts each with two sporozoites (see typical life history p. 418). Agametes without sexual dimorphism. Many species varying in minor details but described by earlier observers as distinct genera which were brought together by Lühe as sub-genera of the genus *Eimeria*, A. Schn. (*Coccidium*, Leuckart).

6. Genus *Eimeria*, A. Schn. (1875). Types of the different sub-genera are given below with the sub-genus name in brackets.
   (*a*) *Eimeria* (*Goussia*, Labbé), Schaudinn (1900). Membrane of sporocyst formed after fertilization; sporoblasts without the unexplained phase known as the "pyramid stage;" sporoblasts spherical or oval with two valves which open in the gut of a new host; parasites of fish (Labbé) and of centipedes (Schaudinn). (See Fig. 178, p. 419.)

(*b*) *Eimeria* s. str. (*Eimeria*, A. Schn.). Membrane of sporocyst formed after fertilization, sporoblasts spherical or oval with a micropyle, opening only in gut of new host. *E. falciformis*, Eimer (1870) parasite of the mouse, *E. stiedæ*, Lindemann (1865), parasite of the rabbit and occasionally of man, cause of acute diarrhea in cattle.

(*c*) *Eimeria* (*Orthospora*, A. Schn., 1881), *salamandræ* (Steinhaus), *propria* (A. Schn.) from different species of Triton, and *ranarum* from the frog. The macrogamete forms the future sporocyst membrane before fertilization, "pyramid stage" absent, sporoblasts spherical or oval opening only in the new host.

(*d*) *Eimeria* (*Paracoccidium*, Laver. and Mesnil, 1902). Membrane of sporocyst formed after fertilization, pyramid stage absent, sporoblast capsules formed but soon dissolved leaving sporozoites free in the sporocyst. *E. prevoti*, Lav. and Mes., in gut of frog.

(*e*) *Eimeria* (*Crystallospora*, Labbé). Sporoblasts crystalline in form of a double pyramid. *E. crystalloides*, Thelohan, 1893. Parasite of the intestine of different species of Motella.

In this genus also are probably to be included *Eimeria avium* Silvestrini and Rivolta, the cause of destructive epidemics of poultry, *E. truncata* Railliet and Lucet (1891) of geese, sporogony alone is known, *E. pfeifferi*, Labbé (1896), parasite of pigeons, *E. faurei*, Moussu and Marotel (1902), of sheep, *E. mitraria*, Lav. and Mes (1902), parasite of *Damonia reevesi*.

Sub-family 6. **Barrouxinæ**, Léger (1911) Fig. 184, A, B, C. The zygotes in this group form many sporoblasts each containing one sporozoite. Several genera have been described but we follow Mesnil and Doflein in regarding them as sub-genera.

7. Genus *Barrouxia*, Schn. Types of the different sub-genera are given below with the sub-genus name in brackets.

(*a*) *Barrouxia* (*Diaspora*, Léger). Sporoblast oval with micropyle.

(*b*) *Barrouxia* (*Barrouxia*, Schn. s. str.). Sporoblast lenticular, bivalved, and smooth, digestive tract of myriapods.

(*c*) *Barrouxia* (*Echinospora*, Léger). Sporoblast oval, bivalved and spinous.

(*d*) *Barrouxia* (*Urobarrouxia*, Mesnil). Sporoblast bivalved with tail-like appendage at each pole.

Sub-family 7. **Caryotrophinæ**, Léger (1911). These are coccidia with a rather complicated asexual cycle involving the formation of many agamete-forming centers termed "agametoblasts." Pseudoconjugation unknown, microgamete-forming centers are derived in the same manner as the "agametoblasts," many sporoblasts are formed, each with many sporozoites, sporoblasts with micropyles.

8. Genus *Caryotropha*, Siedlecki (1902). Parasite of the body cavity of the marine annelid *Polymnia nebulosa*. Frequently groups of individuals are present in a cell, these are either agametoblasts each of which would form a group of agametes, or they are microgametocytes each of which would form a bundle of microgametes. The zygote produces about twenty globular sporoblasts each with about twelve sporozoites.

9. Genus *Klossiella*, Smith and Johnson (1902). Parasite of the mouse kidney, forms found in a glomerulus were interpreted as asexual stages, while those found in the cells of the tubules were regarded as sexual. The full life history is not yet known.

Sub-family 8. **Angeiocystinæ,** Léger (1911). A single genus represents this group at the present time, the individuals are elongate and similar to *Orcheobius* (see above), the zygotes form four sporoblasts each with numerous (up to thirty) sporozoites.

10. Genus *Angeiocystis,* Brasil (1904). Parasite of marine annelid *Cirratulus.*

Sub-family 9. **Leucocytozoinæ,** Doflein (1915). The single genus *Leucocytozoön,* Danilewsky is a comparatively frequent parasite of birds where it is found in the peripheral blood as elongate macro- and microgametocytes in leukocytes. It has been variously placed in classification but its many coccidia-like phases justify Doflein's decision to include it here.

11. Genus *Leucocytozoön,* Danilewsky. Blood of different types of birds.

Sub-family 10. **Aggregatinæ,** Doflein (1916). These are complex forms of Eimeriidæ in which the life history involves a change of hosts. The asexual cycle including agamogony occurs in the gut wall of crabs, here large numbers of agametes are formed, these, with the crabs are eaten by cephalopod molluscs in the intestinal walls of which they develop into gametocytes. Many biflagellated microgametes are formed, the zygote forms many sporoblasts each forming a small number of sporozoites (three, eight, twelve).

Dobell (1925) finds that reduction in number of chromosomes occurs in *Aggregata* immediately after fertilization and not during the formation of the gametes. This agrees with Jameson's account of reduction in the gregarine *Diplocystis scheideri,* Kunstler.

12. Genus *Aggregata,* Frenzel (1885). Several species in different decapods and in cephalopod molluscs.

Order 2. **Hæmosporidia,** Danilewsky, em. Doflein.

The Hæmosporidia are Coccidia-like forms specifically adapted for parasitic life in the blood, particularly of the erythrocytes, although some forms become intracellular parasites of the inner organs. Vertebrates of all classes—mammals, birds, reptiles, amphibia and fish—are subject to infection by one type or other and man is particularly susceptible, the malarial organisms causing serious human diseases which in the tropics are frequently fatal.

Hæmosporidia are minute forms, particularly in the agamous stages during which they frequently show highly motile amœboid forms, but in other cases they are more rigid and appear like the hæmogregarines. Contractile vacuoles are absent but cytoplasmic non-contractile vacuoles, probably connected with nutrition, are characteristic. Pigmented granules (Melanin) are also characteristic and are formed as a product of hæmoglobin break-down and liberated only at periods of reproduction. Other products of metabolism, in the form of toxins, may be liberated at the same time.

Alternation of asexual and sexual generations is the rule, the former taking place in the blood of vertebrates, the latter in the

digestive tract of some blood-sucking arthropod, insects in particular. The prevailing opinion is that arthropods were the primary hosts and that parasitism in the blood is the result of adaptation. One such adaptation, and a very essential one, is the absence of protective capsules about the sporozoites. The latter are always formed in the primary or invertebrate host and are transmitted to the vertebrates at the time of drawing blood. A sporozoite penetrates an erythrocyte and grows to an agamont which forms multiple agametes after a definite interval; these agametes are liberated into the blood where other erythrocytes are entered and the asexual cycle is repeated. The parasites thus multiply rapidly by geometrical progression until enough blood elements are destroyed to

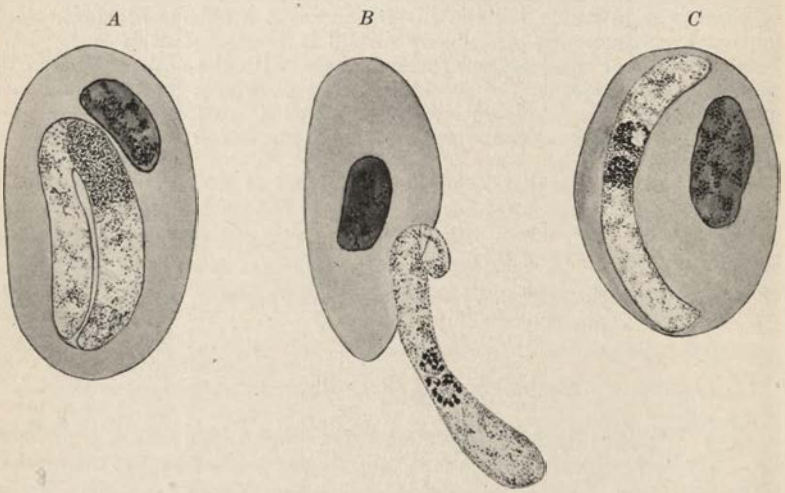

FIG. 185.—Type of Hæmogregarines. *A*, *Hæmogregarina stepanowi*; *B*, and *C*, *Lankesterella ranarum*. (Original.)

produce the first marked symptoms of the infection. Hegner and Taliaferro (1924) estimate about 150,000,000 parasitized blood elements at this time in the case of human malaria, all parasites, if derived from a single infection, undergoing sporulation at practically the same time and liberating their toxin simultaneously into the blood. The pyrexial attacks of chills and fever in human malaria are thus accounted for. Ultimately the agametes develop into gamonts which are usually easy to distinguish from the agamonts and which are frequently differentiated into macrogametocytes and microgametocytes. The gametocytes are taken with the blood into the digestive tract of an invertebrate host (mosquitoes) where the microgametes are formed and where union of gametes occurs. The zygote, like that of some hæmogregarines, is motile and makes

its way by gregariform movement to the wall of the gut. These motile zygotes, termed oökinets by Schaudinn, either enter the epithelial cells of the gut or penetrate them and come to rest against the inner membranes of the gut wall. Here a delicate sporocyst membrane is formed and the amphinucleus divides repeatedly without cytoplasmic division until a vast number of nuclei results. The cytoplasm then divides to form as many naked sporozoites as there are nuclei. The delicate sporocyst membrane is ruptured and the sporozoites are liberated into the body cavity from which they are passed into the blood of the vertebrate and the cycle repeated.

The life cycle of the hemosporidian thus has many points of resemblance to that of the coccidian; the same intracellular mode of life, the same asexual generation and agamete formation, the same formation of gametocytes and dimorphic gametes. The microgametes, however, have no flagella as a rule but move like spirochetes and the zygote, as noted above, forms naked sporozoites. In many cases, however, there is a reminiscence of sporoblast formation, when, after the amphinucleus has divided for a certain limited number of times, the cytoplasm separates into the same number of sporozoite forming centers. The resemblance to the coccidian would be complete if such centers were provided with definite capsules.

Classification of the Hæmosporidia has not yet been perfected. Many blood parasites are minute and their life histories are incompletely known so that even their protozoan affinities are questionable. Such forms will be briefly treated in an appendix to the present chapter. The classification adopted here is not original nor final but is frankly based on expediency, although two of the families—Plasmodidæ and Babesidæ—will probably stand.

Family 1. **Hæmoproteidæ,** Doflein (1916). These are blood parasites of birds showing some of the characteristics of the hemogregarines. There is no apparent asexual increase in the blood cells in which the parasites store up pigment and develop into gametocytes which are earlier known as *Halteridium*. The gametes are formed in the gut of a biting fly such as Lynchia which is louse-like and creeps about in the plumage of birds. Zygotes are formed in the gut of the insect (or may be formed on the slide as first observed by MacCallum, 1898) and as zygotes make their way through the stomach wall into the body cavity of the fly. Here they do not develop further but are transmitted by the bite of the fly to the bird host where metagamic divisions and sporozoite formation do not take place in blood cells but in endothelial cells of the host (Aragao) or in leukocytes. The sporozoites enter erythrocytes and grow into large halter-shape parasites without displacing the nucleus of the cell. These are the gametocytes which are early differentiated as macro- and microgametocytes.

1. Genus *Hæmoproteus*, Kruse. Several species in birds of different groups, *H. noctuæ* in the little owl *Glaucidium noctua; H. columbæ* Celli and Sanfelice in pigeons, *H. danilewskyi*, Grassi and Feletti (1890), in larks, ravens, etc.

Family 2. **Plasmodidæ,** the malaria organisms. These are blood parasites of birds and man causing malarial fevers. Agametes are formed in the red blood cells as are also the gametocytes. The latter mature in the stomach of the mosquito (species of *Culex* and *Anopheles*), zygotes are formed which penetrate the wall of the stomach and form sporocysts against the subepithelial tissue. Here, within a delicate membrane, the sporozoites are formed in groups resembling sporoblasts. The sporozoites, liberated into the body cavity of the mosquito, are transmitted with fluids from the salivary gland to the blood stream of the vertebrate host. Blood parasites of bats and of reptiles are also included here. All have the common property of forming pigment (melanin) at the expense of the hæmoglobin.

2. Genus *Proteosoma*, Labbé (1894). The cause of bird malaria, transmitted by different species of the mosquito family Culicidæ. Widely distributed as bird parasites throughout the world. Probably several species but not easily distinguished from the best known form *Proteosoma præcox* of Grassi and Feletti (1890).
3. Genus *Plasmodium*, Marchiafava and Celli (1885). The human malaria organisms best known by the common names of tertian fever parasites causing pyrexial attacks every third day, quartan parasites causing attacks every fourth day, and tropical fever parasites causing attacks daily or irregularly. Transmission by mosquitoes of the family Anophelidæ. Characteristics of the family. Different species are recognized according to the clinical history or by differences in the gametocytes. Thus *P. vivax*, Grassi and Feletti (1892), is the cause of tertian fever, *P. malariæ* Laveran (1883), the cause of quartan fever and *P. falciparum*, Welch (1897), the cause of tropical fever. In the last species the gametocytes are large and crescentic and differ from the gametocytes of other species and for this reason together with the clinical differences, the species is regarded by some authorites as having generic value and has been named by Grassi and Feletti *Laverania malariæ* in honor of the original discoverer.

   In addition to man and birds other types of mammals are subject to blood infection by species of *Plasmodium*. Monkeys in particular are subject to infection, so also are bats (Dionisi) and squirrels.
4. Genus *Achromaticus*, Dionisi (1898). Questionably included here until the life history is known. The sporozoites form rings in the red blood corpuscles of the bat (*Vesperugo* species) and give rise to four agametes, pyriform cells resembling Babesia (see below). Primary host unknown although Neumann believes he has found developmental stages in the bat louse *Pteroptus vespertilionis*. It differs from other Plasmodidæ in the absence of pigment.
5. Genus *Hæmocystidium*, Castellani and Willey (1904). Blood parasites of reptiles with pigment formation. Agametes two or sometimes four in number are formed in the peripheral blood, gamogony unknown, primary host unknown, included here provisionally (see Shortt, 1922).

Family 3. **Babesiidæ.** These are unpigmented parasites of small size found in the blood corpuscles of various mammals and transmitted by ticks. Amœboid, circular and ring form, and pyriform stages have been described for the same organism. Agamogony by binary division, budding, or multiple division. There is little actual knowledge of the sexual phases in the tick, if they exist, but there is much speculation regarding them. Many genera have been described but until the life history is fully known it seems better to regard them as sub-genera of the original genus *Babesia* of Starcovici, 1893.

6. Genus *Babesia*, Starcovici, 1893.
   (*a*) *Babesia* s. str. Parasites of dogs, cattle (Smith and Kilbourne (1893), *et al.*), sheep (Babes, 1888, Starcovici, 1893), horses (Laveran, 1901), rats (Fantham, 1906), and monkeys (P. H. Ross, 1905).
   (*b*) *Babesia* (*Theileria*, Theiler, 1910). Comma- or rod-shape forms in the red blood corpuscles of cattle in Africa. Very small ($3\,\mu$ long by $\frac{1}{2}\,\mu$ wide) with nucleus in the form of a homogeneous granule of chromatin at one end of the rod. Young forms introduced by bite of the tick (species of the genus *Rhipicephalus*) develop, not in the peripheral blood but in the inner organs, particularly the spleen and lymph glands. Here they undergo multiple reproduction. According to Gonder (1906) gametocytes are formed in the peripheral blood and fertilization occurs in the body of the tick. The zygote then develops as in *Plasmodium*.
   (*c*) *Babesia* (*Anaplasma*, Theiler, 1910). Very minute corpuscular parasites of cattle and dogs, $\frac{1}{2}\,\mu$ in diameter occupying a position near the periphery of the corpuscle. Tick transmission.
   (*d*) *Babesia* (*Nuttalia*, Laveran, 1899). Minute spherical pyriform or rod-shape parasites of the red blood corpuscles of horses, mules, donkeys and zebras in various parts of the world.
   All of these above forms agree in causing serious epidemics in domesticated animals, usually taking the form of hæmoglobinuria or red-water, with marked fever.
   (*e*) *Babesia* (*Toxoplasma*, Nicolle and Manceaux, 1908). A somewhat different type of organism found in the leukocytes and cells of the spleen, liver, kidney, lungs, etc., of rodents, rabbits and dogs: oval, spherical or reniform in shape, multiplication by longitudinal division in cells. França (1917) created a special family for these parasites under the name of *Toxoplasmidæ*.

Still more uncertain in systematic position are the following, some of which are regarded as Protozoa largely because they cannot be placed elsewhere.

Genus *Bartonella*, Tyzzer, Brues, Sellard and Gastiaburu (1915). Coccus or rod forms of parasite in red blood corpuscles, endothelial cells of lymph glands and spleen of man causing Oroya fever in Peru. The spherical bodies appear to be agamonts containing granules and break up into as many rod-shape bodies as there are granules which are supposed to invade other corpuscles. Transmission unknown.

Genus *Rickettsia*, Rocha-Lima (1916). Minute rod-like bodies found in man and transmitted by body lice. Regarded as the cause of trench fever (*R. pediculi*) and of typhus fever (*R. prowazeki*).

Genus *Dermacentroxenus*, Wolbach (1919). The cause of Rocky Mountain spotted fever. Transmitted from ground squirrels by ticks of the genus *Dermacentor*.

Class II. **NEOSPORIDIA**, Schaudinn.

Some authorities (Hartmann, *et al.*) regard the Neosporidia as an independent stem of the Protozoa with close affinities to the Rhizopoda with which they certainly have many common characteristics. They are amœboid and, in the adult stage usually multinucleated. Encapsulated sporoblasts and general mode of life as parasites show some resemblance to the Telosporidia but the

life cycle is less complicated and sexual dimorphism and change of hosts are absent. Unlike the Telosporidia reproduction does not bring the life of an individual to an end but takes place more or less continuously throughout the trophic stages, the sporoblasts being carried about with the more or less active organism which ultimately may become a relatively huge mass of spores.

Sporulation is entirely different from that in the Telosporidia and does not result from the metagamic divisions of a zygote. In a typical form of Myxosporidia in which the amœboid body is multinucleated and the nuclei frequently dimorphic sporulation begins with a peculiar process of internal budding. An island of protoplasm is formed about two of the nuclei, one of each kind if dimorphic, and this island, termed a pansporoblast by Gurley (1893) is equivalent to a sporocyst and gives rise to two sporoblasts each with 7 nuclei after the two nuclei have divided to form 14 nuclei which are now all alike. Two of these 7 nuclei disappear with the formation of the bivalved capsule of the sporoblast; 2 of them disappear with the formation of peculiar nematocyst-like capsules termed polar capsules containing coiled threads, 1 is cast out of the cell and 2 remain as the sporoblast nuclei which sooner, or later, unite to form one a process of fertilization known as autogamy (Fig. 186).

The complications are thus of quite a different character from those of the Telosporidia but they are not shown by all of the Neosporidia, polar capsules, for example, being absent in Sarcosporidia.

The group is so highly diverse that generalizations are impossible apart from the very general statement that, like Telosporidia, they may be lumen-dwelling (cœlozoic), tissue-dwelling (histozoic) particularly of the muscle tissues, or cell-dwelling (cytozoic) in habitat, and that new hosts are invariably infected by the contaminative method, usually by way of the digestive tract.

Compared with the Telosporidia the life histories and cytology of Neosporidia are little known. Morphologically, however, more advance has been made and enough to warrant the division into two sub-classes—Cnidosporidia and Sarcosporidia, with an outline classification as follows.

Class Neosporidia, Schaudinn (1900).
- Sub-class 1. Cnidosporidia, Doflein.
  - Order 1. Myxosporidia, Bütschli.
    - Sub-order 1. Eurysporea, Kudo.
      - Family 1. Ceratomyxidæ, Doflein.
    - Sub-order 2. Sphærosporea, Kudo.
      - Family 1. Chloromyxidæ, Thelohan.
      - Family 2. Sphærosporidæ, Davis.

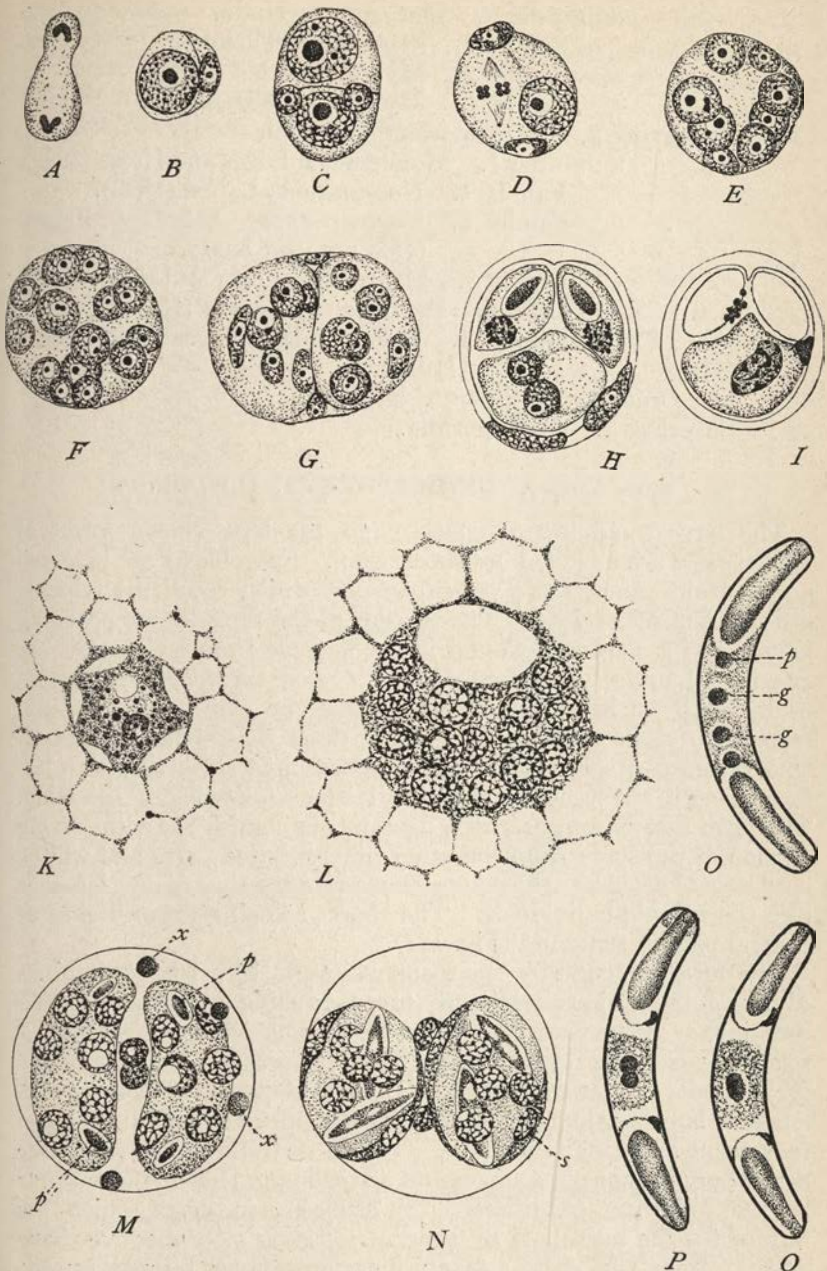

FIG. 186.—*Myxobolus pfeifferi* (*A* to *I*) and *Sphæromyxa sabrazesi* (*K* to *Q*). (See text). (After Keysselitz and Schröder.)

Sub-order 3. Platysporea, Kudo.
Family 1. Myxidiidæ, Thelohan.
Family 2. Myxosomatidæ, Poche.
Family 3. Myxobolidæ, Thelohan.
Order 2. Microsporidia, Balbiani.
Sub-order 1. Monocnidea, Léger and Hesse (1922).
Family 1. Nosematidæ, Labbé (1899).
Family 2. Coccosporidæ, Léger et Hesse (1922) emend Kudo.
Family 3. Mrazekidæ, Léger and Hesse (1922)
Sub-order 2. Dicnidea, Léger and Hesse (1922).
Family 1. Telomyxidæ, Léger and Hesse (1910).
Order 3. Actinomyxida, Stolç.
Sub-class 2. Sarcosporidia.

Sub-class I. **CNIDOSPORIDIA**, Doflein.

The largest number of species and the best known forms of Neosporidia are included in this division. Sporoblasts are bivalved and contain one or more polar capsules which recall the stinging cells of the Cœlenterata. The threads of the capsules are probably hollow and are spirally wound in the capsule from which they are evaginated under proper conditions. Such threads, the function of which is entirely problematical, may be short or very long, reaching in some cases a length many times that of the sporoblast. The germs can scarcely be called sporozoites since they are not formed as a result of metagamic divisions following fertilization. The term sporoplasm has been used to distinguish the vital, living portion of the spore from the other differentiated parts and will be used here to designate the young germ up to the time of development into the trophic individuals. The spores are all built on the same general plan of structure (Fig. 187).

The form assumed by the trophozoites varies with the habitat. Many of the Cnidosporidia are lumen-dwelling and many are cell-dwelling, or tissue parasites. The free forms are characterized by relatively complex organization with ectoplasm, endoplasm and pseudopodia similar to amœbæ. The pseudopodia may be filiform, lobose or lamellate and locomotion is frequently as active by amœboid movement as in many amœbæ. Tissue- or cell-dwelling forms are active only in the young stages and according to Doflein may appear in the following conditions: (1) Enclosed in cysts which are formed for the most part by concentric layers of connective tissue derived from the host, and an innermost layer formed by the organism. Huge cysts resulting from association of parasites, and easily visible to the naked eye are formed in many cases. (2) "Diffuse infiltration" a term used to indicate collections of parasites

between tissue cells where they may fill up cavities without doing much or any harm to the host. (3) Intracellular parasites whereby the usually minute organisms live at the expense of the cell host. The life histories of different types will be given in connection with the different groups as no general account will suffice for all.

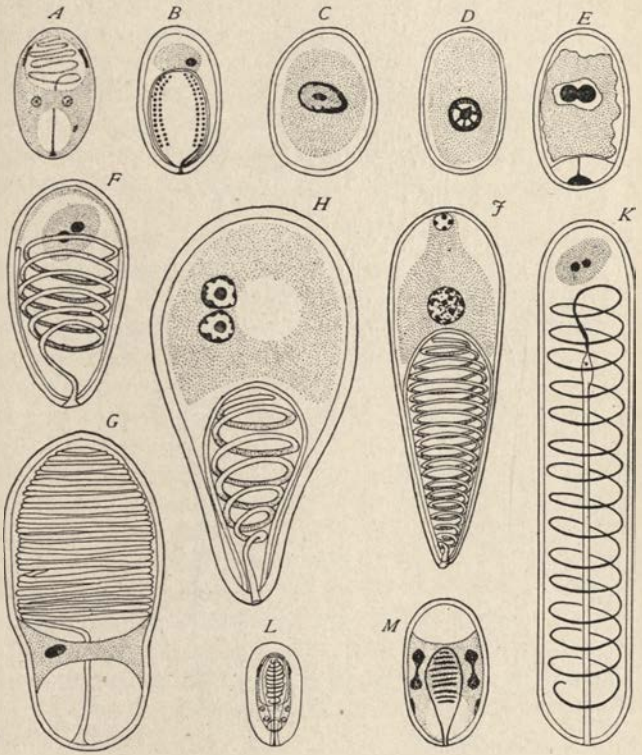

FIG. 187.—Types of Cnidosporidian spores. *A*, *Nosema apis*. After Fantham and Porter; *B*, same, after Kudo; *C*, *D*, *E*, different Haplosporidia spores, after Swellengrebel, Perrin, and Swarczewsky; *F*, *Plistophora macrospora*, after Léger and Hesse; *G*, *Plistophora longifilis*, after Schuberg; *H*, *Myxobolus toyamai*, after Kudo; *J*, *Stempellia magna*, after Kudo; *K*, *Mrazekia argoisi*, after Léger and Hesse; *L*, *Nosema bombyces*, after Stempel; *M*, *Thelohania giardi*, after Mercier. (From Kudo.)

ORDER 1. **Myxosporidia,** BÜTSCHLI.

The Myxosporidia are the best known of the Neosporidia both as to number of species and life histories. Of the 249 species listed by Kudo (1919) all but 11 are parasitic in fishes, 5 have been found in amphibia, 4 in reptiles, 1 in an insect and 1 in an annelid. They are, therefore, characteristic fish parasites, where they occur both as cœlozoic and as histozoic forms, never, according to Davis (1917),

in the digestive tract, but the free forms mainly in the gall and urinary bladders, the tissue parasites mainly in the connective and muscular tissues. The free forms produce no evident harmful effects

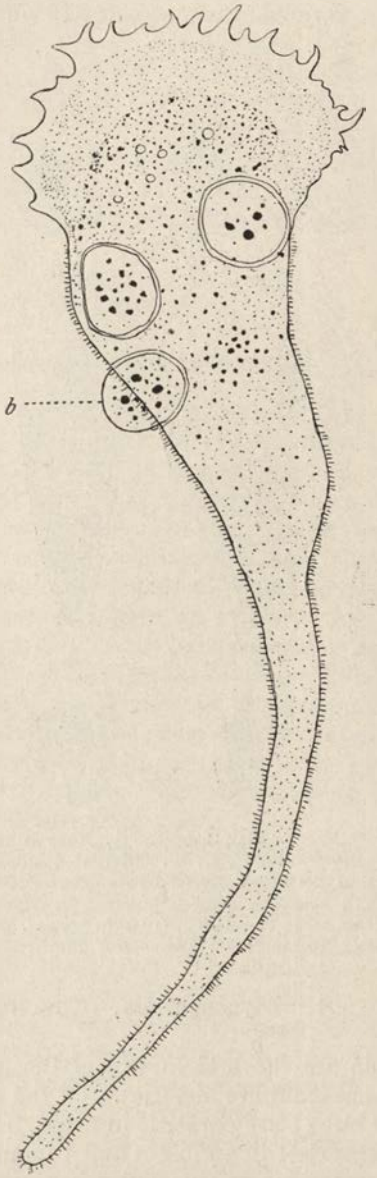

FIG. 188.—Internal buds or "gemmules" of *Sphærospora dimorpha*, a myxosporidian. (After Davis.)

on the host but the tissue parasites are more disastrous, *Myxobolus pfeifferi*, for example, causing costly epidemics amongst food fishes, particularly in the barbel (*Barbus barbus* L.) of Europe.

The free or cœlozoic forms are the most generalized in structure and the tissue parasites are generally regarded as having been derived from them by adaptation (Auerbach, 1910; Doflein, 1916; Davis, 1917, *et al.*). They are somewhat more numerous than the tissue-dwelling forms, Kudo enumerating 125 species of the former and 114 of the latter while 3 species are apparently transitional, and 7 of unknown habitat. The free forms often show a remarkable resemblance to amœbæ; ectoplasm and endoplasm are usually differentiated, the former as in some amœbæ, forming a continuous cortical zone about the organism, or as in other types of amœbæ, evident in certain regions only (Fig. 188). It is occasionally provided with bristle-like processes and the pseudopodia of different types are invariably derived from it (Davis).

The endoplasm is more fluid than the ectoplasm, contains many nuclei and metaplasmic bodies in the form of fat globules, pigment granules, and crystalline bodies, in some cases embedded in structures which under the name of spherules (Davis) are sometimes so abundant as to give a characteristic appearance to the organism (Fig. 189).

Like other Sporozoa, the Myxosporidia are highly prolific and adaptations to this end are well marked. Asexual reproduction occurs by simple division or by multiple division (plasmotomy), and by budding. Exogenous budding described by Cohn, 1896 in *Myxidium lieberkühni* is regarded by Davis (1916) as abnormal and without significance in reproduction but internal or endogenous budding occurs in *Sinuolinea dimorpha*, Davis, where free cells are formed about nuclei in the endoplasm. These cells, called "gemmules" by Davis escape from the parent organism and develop into individuals (Fig. 188).

Propagative reproduction involves the formation of spores and the nearest approach to sexual processes to be found in the Neosporidia. The process has been described by various observers and the general agreement of these descriptions indicates a common plan throughout the group. Schröder's account of sporulation in *Sphæromyxa sabrazesi*, Lav. and Mes. may be selected as an example for the entire Order. This form is parasitic in the sea-horse *Siphonostoma rondeletii*, and like many others has dimorphic nuclei distinguishable by size and structure. Small areas become differentiated within the endoplasm and contain two nuclei, one of each type. These areas, the so-called pansporoblasts, are the mother cells of the spores. Each nucleus divides in such order that 7 nuclei arise from each; the mother cell then divides into two cells which are destined to form two spores. Each of these cells has

7 nuclei 1 of which is cast out as a "reduction" nucleus; 2 are involved in the formation of the two valves of the spore and ultimately disappear; 2 are connected with the elaboration of the polar capsules and similarly disappear and 2 remain as germinal nuclei. It is generally assumed that these 2 nuclei are descendants of the

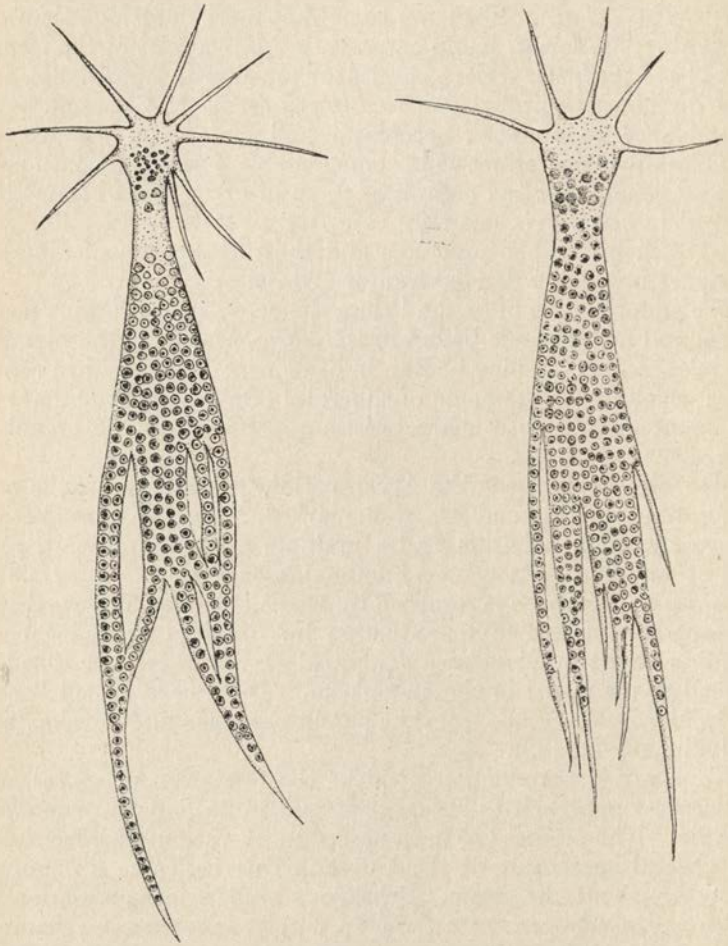

FIG. 189.—*Leptotheca scissura*, vegetative individuals with well-developed spherules. (After Davis.)

original dimorphic nuclei of the trophozoite and observations by Schröder (1910), Davis (1916), Erdmann (1911 and 1917), leave little doubt that they ultimately fuse in autogamous fertilization p. 547).

The spores which differ from sporoblasts of the Telosporidia in

that they are not formed as a result of fertilization, are the most characteristic structures of the Myxosporidia and are much more highly differentiated than are sporoblasts of the former group. They conform to the same general plan of structure throughout but differ in axial relations and in sculpturing, as well as in number and time of appearance. The spore capsule always consists of two valves which are independently developed and come together with a median suture dividing the spore into right and left halves. In different types the spores may be elongated in the plane of the suture or at right angles to it. The polar capsules with their coiled threads indicate what most authorities regard as the anterior end although spores of the Myxidiidæ have thread capsules at each end of the elongated spore (Fig. 187). We agree with Kudo (1919) that the axial nomenclature suggested by Davis (1917) is unnecessarily complicated and that antero-posterior differentiation is characteristic of the vast majority of these spores. Lateral processes, posterior spines, and external sculpturing of various types distinguish the different genera and species and afford a means of classification. In this we follow Kudo (1919) as follows:

Sub-order 1. **Eurysporea**, Kudo (1919).

Largest diameter of the spore at right angles to the sutural plane with one polar capsule at each side of the plane and without "iodinophilous vacuole." Cœlozoic parasites for the most part, the great majority in marine fish. A single spore (monosporous), double spores (disporous) and many spores (polysporous) formed by individuals.

Family 1. **Ceratomyxidæ**, Doflein (1899). The sole family with characters of the sub-order.

1. Genus *Leptotheca*, Thelohan (1895). Marine fish parasites; spore with hemispherical or rounded valves; disporous where known; 15 species.
2. Genus *Ceratomyxa*, Thelohan (1892). Marine fish parasites; valves of spore extended into long lateral conical and hollow processes; sporoplasm asymmetrically placed; monosporous, disporous and polysporous; 35 species.
3. Genus *Myxoproteus*, Doflein (1898) em. Davis (1917). Marine fish urinary bladder; spores more or less pyramidal; disporous; three species.
4. Genus *Wardia*, Kudo (1919). Tissue parasites of fresh-water fish and amphibia; spore an isosceles triangle with convex sides; surface of spore with fine ridges with fringe of processes at the posterior end; polysporous; 2 species.
5. Genus *Mitraspora*, Fugita (1912) em. Kudo. Fresh-water fish kidney, spores spherical or ovoidal, valves longitudinally striated with or without long, fine filaments projecting posteriorly in a row at right angles to the sutural plane at the posterior end, disporous and polysporous; 3 species.

Sub-order 2. **Sphærosporea**, Kudo (1919).

Spores spherical or subspherical with two to four polar capsules; sporoplasm without iodinophilous vacuole; monosporous, disporous and polysporous; body cavity and tissues of fresh- and salt-water fish and amphibia.

Family 1. **Chloromyxidæ**, Thelohan (1892). Spores with four polar capsules; one, two, or many spores.

6. Genus *Chloromyxum*, Mingazzini (1890). With the characters of the family, 22 species, 2 in amphibia, 20 in fresh- and salt-water fish.

Family 2. **Sphærosporidæ**, Davis (1917). Spores with two polar capsules, monosporous, disporous and polysporous.

7. Genus *Sphærospora*, Thelohan (1892). Cœlozoic and histozoic parasites of fresh- and salt-water fish, spores with two polar capsules, 10 species.
8. Genus *Sinuolinea*, Davis (1917). Urinary bladder of marine fish, spores with or without lateral processes, disporous and polysporous, sutural line of spore sinuous, 5 species.

Sub-order 3. **Platysporea**, Kudo (1919).

Sutural plane the longest diameter of the spore or at an acute angle with it; one or two polar capsules; sporoplasm with or without an iodinophilous vacuole. Fusiform.

Family 1. **Myxidiidæ**, Thelohan (1892). Spore with two polar capsules, one at each end; sporoplasm without iodinophilous vacuole.

9. Genus *Myxidium* Bütschli (1882). Cœlozoic and histozoic parasites of salt- and fresh-water fishes and in reptiles; spores more or less regularly fusiform with pointed or rounded ends; polar threads long and fine, monosporous, disporous and polysporous, 26 species.
10. Genus *Zschokkella*, Auerbach (1910). Cœlozoic parasites of fresh- and salt-water fish, spores semicircular in front view, polar capsules large and spherical, sutural line usually S-form, monosporous, disporous and polysporous, 4 species.

Family 2. **Myxosomatidæ**, Poche (1913). Spores with two polar capsules at the anterior end, sporoplasm without iodinophilous vacuole.

11. Genus *Myxosoma*, Thelohan (1892). Histozoic parasites of fresh- and salt-water fish, spores ovoidal, flattened, and more or less elongate, polysporous, 3 species.
12. Genus *Lentospora*, Plehn (1905). Histozoic parasites of fresh- and salt-water spores lenticular in form with two polar capsules at the anterior end, disporous and polysporous, 6 species.

Family 3. **Myxobolidæ**, Thelohan (1892). Cœlozoic and histozoic parasites of fresh-water fishes mainly, spores with one or two polar capsules at the anterior end and with or without posterior processes, sporoplasm with an iodinophilous vacuole, mainly polysporous.

13. Genus *Myxobolus*, Bütschli (1892). Histozoic parasites of fresh-water fish (56 species), marine fish (5 species), annelids (1) and amphibia (1), spores ovoidal, ellipsoidal or flattened, with one or two polar capsules, without posterior processes, 63 species.
14. Genus *Henneguya*, Thelohan (1892). Cœlozoic and histozoic parasites mainly in fresh-water fish, spores spheroidal or ovoidal with two polar capsules at the anterior end, posterior ends of shell valves prolonged into processes which unite to form a tail in the median line, monosporous, disporous and polysporous; 32 species.
15. Genus *Hoferellus*, Berg (1898). Cœlozoic and histozoic parasites of fresh-water fish; spores pyramidal with two posterior processes from the lateral faces; polysporous, 1 species.

Order 2. **Microsporidia,** Balbiani.

Probably because of their minute size the organisms included in this Order are incompletely known and many points of structure and of life history are still unknown or controversial. They are practically all cell parasites which enter the host by way of the digestive tract from which they may spread to all tissues of the body, causing epidemics not only in fish, but economically more important, costly epidemics in silkworms (*Nosema bombyces*, Naeg.) and honey bees (*Nosema apis*, Zander). Pseudopodia and amœboid movement are rarely observed (*Nosema marionis*, Thel). Intermediate hosts are unknown.

Agamous reproduction is well established through the observations of many investigators. The agametes are small, uninucleate, and usually with indefinite outlines which scarcely delimit them from the host cell protoplasm; they may have one or several nuclei, and multiply actively by simple division resulting frequently in chain formation through successive nuclear divisions and delayed cell division (Fig. 190). As a result of such agamous reproduction all of the tissues of the host may become infected and myriads of tissue cells destroyed. In many species tumor-like masses are formed in which the organisms are surrounded by a membrane derived from the host and are thus encapsulated; in other species such membranes are absent. In the majority of cases spread of the infection in the same host comes to an end with sporulation, but in some species renewed infection is brought about by the action of the digestive fluids on spores formed in the same organism (Kudo).

Multiple endogenous budding, or fragmentation of the trophozoite into numerous binucleate agametes is described for some forms (Debaisieux, 1920) and these, as in Telosporidia, ultimately give rise to the sporulating individuals. The phenomena of sporulation differ widely but there is still much uncertainty in the different accounts at hand. In some cases the trophozoites are said to produce pansporoblasts as in Myxosporidia during the continued vegetative life of the individual (Polysporea). Such cases included formerly under the name Blastogenea, are regarded as very doubtful by Doflein (1916, p. 1037). In other cases the trophozoite (pansporoblast?) breaks up into numerous sporulating cells each of which produces one or more spores (Oligosporea) and in still other cases, the entire individual forms a single spore without pansporoblast formation (Monosporea). The absence of pansporoblasts in such cases is regarded as evidence of extreme adaptation on the part of the exclusively cytozoic parasites (*Nosema* species).

The spores on the whole are less complex than those of the Myxosporidia. They are small and ovoidal or bean-shape and rarely (*Telomyxa*, Léger and Hesse, 1910) with more than one polar capsule,

in some cases without any. The capsules and threads are invisible or very difficult to see in the living spore (hence cryptocysts), but are demonstrable upon treatment with alkalies. The spore capsule is bivalved in some but consists of a single piece in other species. The history of spore-formation agrees in the main with that of the

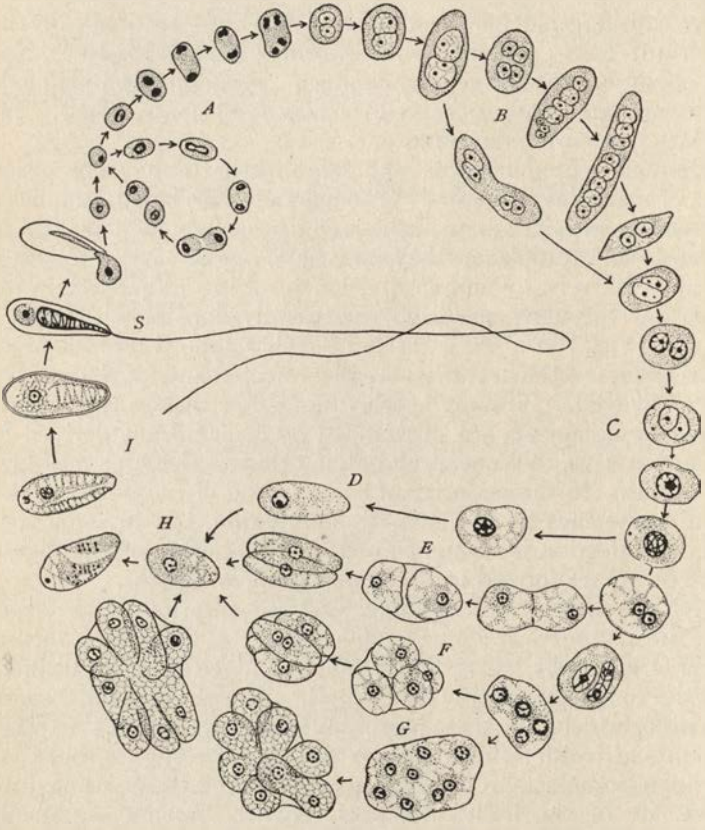

FIG. 190.—*Stempellia magna*, life cycle. *A*, Developmental stages of young amœbula from spore S.; *B*, stage of nuclear increase; *C*, formation of sporont; *D*, formation of a single spore, *E*, formation of two spores; *F*, formation of four spores; *G*, of eight spores; *H*, development of uninucleated spore with polar capsule. (After Kudo.)

Myxosporidia but authorities disagree as to details and convincing proof is yet to be demonstrated. Fertilization processes have been described by Mercier (1908, 1909) whereby two isogametes of *Thelohania giardi* fuse to form the pansporoblast, an observation which has not been confirmed. Autogamous union of nuclei prior to spore-formation and not, as in Myxosporidia in the later sporo-

plasm, has been described by Debaisieux (1913, 1915) in species of *Thelohania* and *Glugea*.

The life history of *Stempellia magna* as given by Kudo (1924) is typical of the Microsporidia (Fig. 190). The polar filament of the spore (*S*) is extruded when the spore reaches the mid-gut of its culicine host; the uninucleate sporoplasm creeps out of the opening

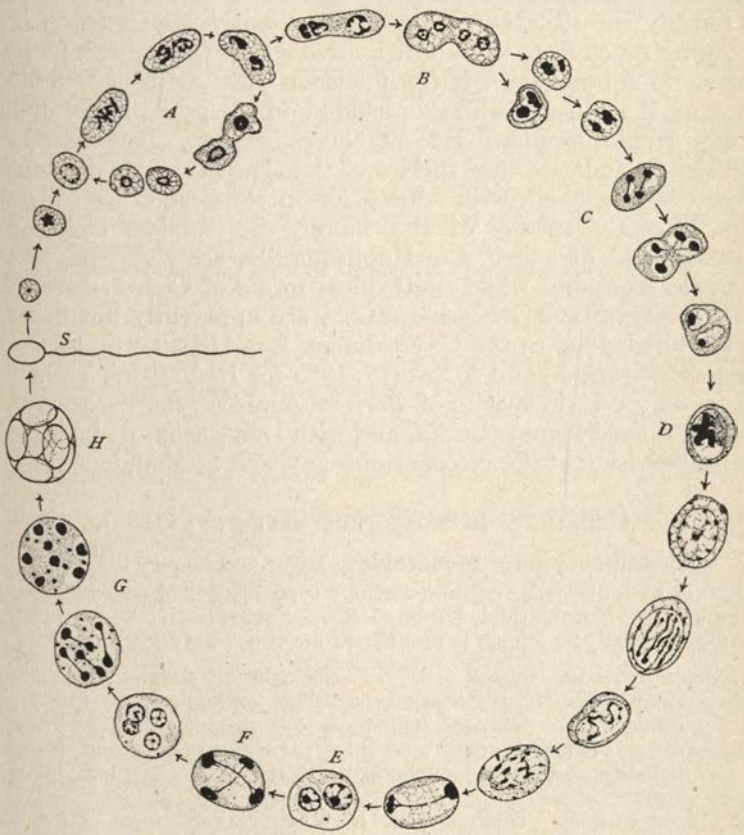

FIG. 191.—*Thelohania légeri*, life cycle. *A*, Early stages of sporozoite after leaving the spore *S*; *B*, formation of binucleated individuals; *C*, repeated binary division; *D*, fusion of the two nuclei to form the sporont; *E*, to *H*, nuclear and cell divisions to form eight sporoblasts each of which forms one spore. (After Kudo.)

made by the cast-off filament, enters a fat cell and becomes an agamont and reproduces by division (*A*). The products ultimately become multinucleated with from four to eight nuclei (*B*), the organisms then breaking up into binucleated cells the nuclei of which fuse after discarding some chromatin (*C*). This is identified as a sporont which may become transformed into a single spore (*D*), or

it may divide into two (*E*), four (*F*), or eight (*G*), sporoblasts each of which forms a single spore after chromidia formation and reconstruction of small nuclei (*H*, *I*), some of which take part in the formation of the capsular thread. A more simple life history is shown by *Thelohania légeri* according to Kudo (Fig. 191).

Classification of the Microsporidia has been in a most unsatisfactory state but recent monographs on the group have done much to remedy the situation. The minor subdivisions given below do not differ much from those which have served the purpose for many years. One innovation is the inclusion amongst Microsporidia of some of the genera hitherto included in a special major division —the Haplosporidia. Recent investigations, particularly by Debaisieux, indicate that species of the type genus *Haplosporidium* should be included with the Microsporidia and are so treated here. Certain species of the Family Bertramidæ included by Caullery and Mesnil in the Haplosporidia, are also grouped with the Microsporidia; while the typical forms of Cœlosporidia, *e. g.*, *Cœlosporidium* and *Rhinosporidium*, are apparently not Sporozoa at all, but belong to the Chytridiaceæ (see Debaisieux, 1916, 1920 for *Cœlosporidium*, and Ashworth, 1923 for *Rhinosporidium*). This leaves only certain species of *Bertramia* and a group of the *incertæ sedis* in the old Haplosporidia, and with Debaisieux, we believe that this subdivision of the Neosporidia may well be abandoned.

Order 2. **Microsporidia**, Balbiani (1882).

Sub-order 1. **Monocnidea**, Léger and Hesse (1922).

Spores with one polar capsule and one typically coiled polar filament.

Family 1. **Nosematidæ**, Labbé (1899). Spores oval, ovoid or pyriform; if subcylindrical the length is less than four times the breadth.

1. Genus *Nosema*, Naegeli (1857). Each sporont develops into a single spore. Widely distributed parasites particularly in insects. *N. bombyces*, the cause of pébrine in silkworms; *N. apis*, Zander, the cause of wide-spread epidemics in honey bees; other species are parasitic in Protozoa, copepods, Diptera and Lepidoptera, crabs and crayfish.
2. Genus *Glugea*, Thelohan (1891). Each sporont forms two spores; host cells hypertrophied; forming so-called glugea-cysts. Muscle and connective tissue of fish, amphibia, annelids and copopods.
3. Genus *Perezia*, Léger and Duboscq. Each sporont forms two spores; host cells not hypertrophied. Parasites in gregarines (*Lankesteria*) and in Lepidoptera.
4. Genus *Gurleya*, Doflein (1897). Each sporont produces four spores; parasites of copepods and insects.
5. Genus *Thelohania*, Henneguy (1892). Each sporont develops into eight sporoblasts, each of which forms one spore. Parasites of copepods, decapods and insects, particularly mosquitoes.
6. Genus *Stempellia*, Léger and Hesse (1922). Sporonts develop into one, two, four, or eight sporoblasts, each sporoblast forms one spore. Parasites of insects.

7. Genus *Duboscqia*, Perez. Each sporont develops into sixteen sporoblasts each of which forms one spore. Parasitic in *Termes lucifugus*.
8. Genus *Plistophora*, Gurley (1923). Each sporont develops into more than 16 spores. Widely distributed parasites in copepods, insects and fish.

Family 2. **Coccosporidæ**, Léger and Hesse (1922), emend Kudo (1924). Spores spherical or sub-spherical. A single genus:

9. Genus *Coccospora*, Léger and Hesse (1922), Kudo (1924). Parasites of oligochetes, insects and crustacea.

Family 3. **Mrazekidæ**, Léger and Hesse (1922). Spores tubular or elongate cylindrical (length more than five times the diameter).

10. Genus *Mrazekia*, Léger and Hesse (1916). Spores straight-tubular; polar filament with rod-like base. Parasites of annelids, crustacea and insects.
11. Genus *Octosporea* Flu (1911), emend Chatton and Krempf (1911). Spores cylindrical; more or less arched; ends similar. Parasites of Diptera.
12. Genus *Spirospora*, Léger and Hesse (1922), emend Kudo (1924). Spores tubular and spirally curved; polar capsule occupies major part of the spore; filament without rod-like base. One species parasitic in Diptera larvæ.
13. Genus *Toxospora*, Léger and Hesse (1922), emend Kudo (1924). Spores very small, curved in semicircle. One species, parasitic in Diptera larvæ.

Sub-order 2. **DICNIDEA**, Léger and Hesse (1922).

Spores with two polar capsules, one at each end; each capsule with polar filament. One family only.

Family 1. **Telomyxidæ**, Léger and Hesse (1910). One genus.

14. Genus *Telomyxa*, Léger and Hesse (1910). Parasite of the larva of *Ephemera vulgata*.

Genera *incertæ sedis*.

15. Genus *Haplosporidium*, Caullery and Mesnil. Oligochete parasite. Many sporoblasts each with four spores.
16. Genus *Serumsporidium*, L. Pfeiffer. Body cavity of Cypris species.
17. Genus *Lymphosporidium*, Calkins. Cavities of brook trout.
18. Genus *Paramyxa*, Chatton. Without polar capsules. Marine annelid.
19. Genus *Blanchardina*, Labbé.
20. Genus *Botellus*, Moniez.
21. Genus *Lymphocystis*, Woodcock.
22. Genus *Bertramia*, Caullery and Mesnil.
23. Genus *Cœlosporidium*, Crawley.

Order 3. **Actinomyxida**, Stolc.

These are Cnidosporidia about which little is known beyond the process of sporulation. In its fully grown condition the entire body may be interpreted as one pansporoblast which is surrounded by a membrane, and which usually produces eight spores, the membranes of which are usually triradiate and drawn out into elaborate

spines. Each spore has three polar capsules containing distinct protrusible filaments.

The development processes leading to the formation of spores involves fertilization phenomena of a characteristic type. They are essentially similar to those of the Myxosporidia but differ in some important details. A plasmodial stage appears to be absent or represented by a binucleate amœbula only, which develops into a spore. The two nuclei divide and form 4 cells, 2 of which disappear with the formation of a membrane within which the other 2 cells lie. Each of these divides forming 4, 2 of which continue to divide rapidly until 8 are formed while the other 2 remain large and

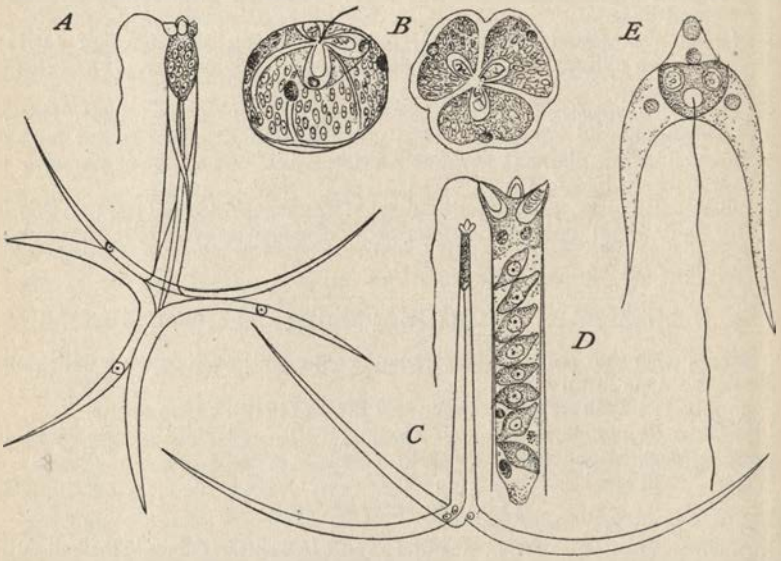

FIG. 192.—Spores of Actinomyxida. *A*, *Hexactinomyxon psammoryctis*, after Stolç, *B*, *Sphæractinomyxon stolsi; C*, *Triactinomyxon ignotum; D*, same, spore bearing part enlarged, after Léger; *E*, *Synactinomyxon tubificis*. (After Caullery and Mesnil.)

undivided the two-celled membrane now containing 8 small and 2 large cells. Ultimately the 2 large nuclei begin to divide in turn until 8 products result and 16 cells, regarded by Caullery and Mesnil (1905) and by Ikeda (1912) as gametes, lie free in the cyst. The two sets of gametes differ slightly in nuclear size and in staining capacity and unite 2 by 2 to form 8 zygotes. The nucleus of each zygote now divides until 6 small nuclei and 1 large one result, the large one destined to form a mass of sporozoites. The 6 small ones arrange themselves in such a manner as to form 3 shell-forming cells, while 3 of them lie within and form 3 polar capsules. The germ-forming cell is not enclosed by the spore-forming cells but lies

outside of it and peripherally in the pansporoblast. It divides repeatedly until 8, 32, or many sporozoites result (Fig. 192).

The Actinomyxida are parasites of annelids and sipunculids and the spores are invariably triradiate. The anchor or star-form processes of the capsule are regarded by Doflein as supports in floating, evidence for which is given by Kofoid's observation of these spores in plankton.

Ikeda (1912) divides the five known genera into two groups which he designates Simplicia, with one genus *Tetractinomyxon*, Ikeda, and Multiplicia with the other four genera.

1. Genus *Tetractinomyxon*, Ikeda (1912). Parasite of the sipunculid *Petalostoma minutum*, Kef. Pyramidal spores with two membranes ecto- and endospores. Three ectospore nuclei at the angles of the base of the pyramid, three polar capsules at the apex.
2. Genus *Hexactinomyxon* Stolç (1899). Spores with six anchor-like processes; parasite of the intestinal epithelium of *Psammoryctes barbatus*.
3. Genus *Triactinomyxon*, Stolç (1899). Spore with three anchor-like processes parasite of intestine of *Tubifex tubifex*.
4. Genus *Synactinomyxon*, Stolç (1899). Spores with two long and one short, sharp-pointed processes; parasites of the intestine of *Tubifex rivulorum*.
5. Genus *Sphæractinomyxon*, Caull. and Mesnil (1905). Spores spherical and without pointed processes, parasites in the body cavity of the marine oligochete *Clitellis arenarius* O. F. M.

Sub-class II. SARCOSPORIDIA.

The Sarcosporidia are parasites of vertebrates, particularly mammals in which the ultimate seat of parasitism is the muscular tissue. There is but one genus—*Sarcocystis*—with several species in pigs (*S. miescheriana*, Kühn, 1865, forming "miescher's tubules"), in sheep (*S. tenella*, Railliet, 1886), in cattle (*S. blanchardi*, Doflein, 1901), in mice (*S. muris*, Blanchard, 1885), in opossums (*S. darlingi*, Brumpt, 1913) in monkeys (*S. kortei*, Castellani and Chalmers, 1909), and in man (*S. lindemanni*, Rivolta, 1878). A species from birds was described by Stiles (1893) under the name of *S. rileyi*.

Sarcosporidia have been studied by a host of observers and an almost equal number of interpretations has been the result. The best known species is *S. muris* from the mouse in which, beginning with Th. Smith's (1901) inoculation experiments by feeding infected tissues to mice, the young stages and their development are now known. Observations made by this method of study, particularly by Erdmann (1910 *a*, *b*, *c*, and 1914), and by Crawley (1914 and 1916) and Marullaz (1920) permit of a tentative life history of *S. muris* as follows.

Infection occurs by eating infected tissues, or as Négre (1907) showed, by eating contaminated feces. The germs, regarded by

Erdmann (1914) as sporozoites, enter the epithelial cells within an hour to an hour and a half (Crawley and Marullaz). Here, according to Crawley (1914 and 1916), they develop directly into gametocytes which are sexually differentiated. The microgametocytes become practically all nucleus the chromatin of which is distributed in groups of granules about the periphery; each group forms a single microgamete, the spermatozoids being arranged about the periphery very much like the microgametes of a coccidian. The macrogametocytes retain most of their cytoplasm and become macrogametes. The latter are fertilized by a microgamete. The zygotes then give rise to a large number of products (the sporoblasts of Erdmann) which may enter the musculature, or may possibly pass out with the feces (Crawley). Here there is a gap in the accounts of the life history but ultimately the muscles are invaded and asexual multiplication results in a number of sporozoites (Erdmann) groups of which are massed together and kept in place by membranes formed by the host. Upon reinfection these develop again to gametocytes.

It is evident that if this account of the life cycle, the important sexual phases of which are supplied by Crawley, is confirmed by further studies, the Sarcosporidia should not be retained in the Neosporidia, but as Crawley suggests, should be placed with the Coccidiomorpha. Until such confirmation is forthcoming the older arrangement is retained.

III. QUESTIONABLE PROTOZOA. CHLAMYDOZOA.

The term Chlamydozoa was applied by Prowazek (1907) to intracellular structures found in human tissues in connection with certain diseases, and regarded by him, as well as by many others, as Protozoa. Others, pathologists particularly, looked upon them as degeneration products of the diseased cells, or as artefacts due to technical processes, and they are still more commonly referred to not by the generic and specific names which they have received, but by the names of the men who first studied them. Thus the "Guarnieri bodies" refer to the characteristic cell inclusions of variola and vaccinia which were named by Guarnieri (1892) *Cytoryctes variolæ* and *Cytoryctes vacciniæ;* the "Negri bodies" similarly refer to the inclusions in nerve cells of animals suffering from hydrophobia and named by Williams and Lowden (1906) *Neuroryctes hydrophobiæ* (Fig. 193). More or less similar inclusions of diseased cells have been described from molluscum contagiosum (Prowazek) trachoma (Prowazek and Halberstædter), epitheliosis desquamativa (Leber and Prowazek), swine pest (Uhlenhuth), sprue (Castellani), bird-pox, sheep-pox, verruga peruviana, herpes (zoster, genitalis, and febrilis). The latter were regarded by

Lipschutz (1912) as organisms having the same attributes as Chlamydozoa but named by him Strongyloplasmata. Other minute organisms which occur in the form of filterable viruses have been included in this group, the Rickettsia species causing Rocky Mountain spotted fever, trench fever and typhus fever for example are so treated by da Rocha-Lima (1916), Jungmann (1919), and others (see p. 445).

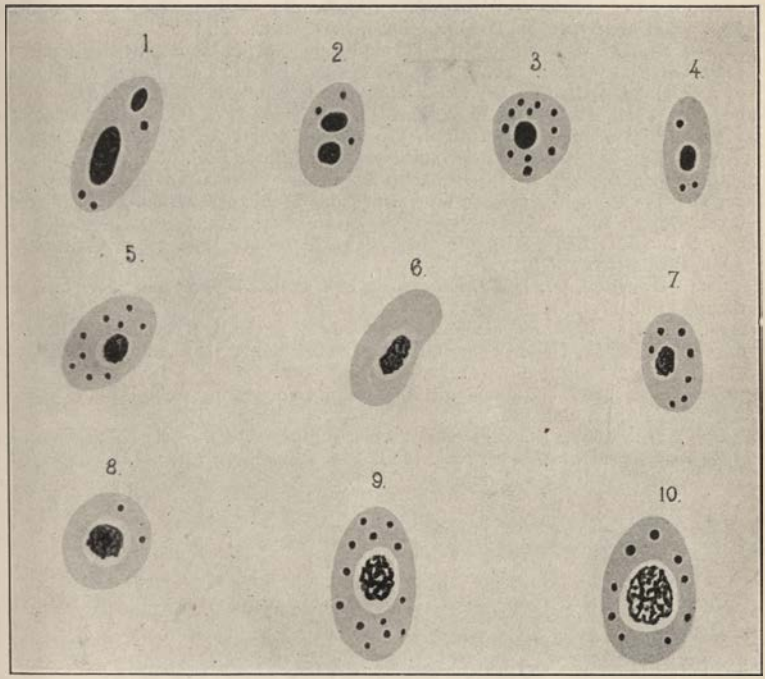

FIG. 193.—"Negri Bodies," *Neuroryctes hydrophobiæ*. Different forms assumed; are they nucleated cells or are the apparent cell-bodies specific secretions of nerve cells of the host about the parasites, thus justifying the term "Chlamydozoa" ? (After Negri.)

The inclusions are in the form of granules of homogenous nature and of very small size and are generally known as the "elementary granules" which become invested in a mantle of substance derived from the nucleus of the host cell hence the name Chlamydozoa or "mantle-covered" animals. The granules are generally regarded as microörganisms but even this is questioned by many (*e. g.*, Cowdry, 1922). Others regard the entire inclusion, mantle and all as the organism (Negri, Councilman, Calkins, Williams, *et al.*) evidence for which is given by Williams, 1906 (Fig. 193). Despite a vast literature on the subject the matter is still unsettled and many still regard the etiology of the diseases in question as unknown.

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CHAPTER X.

VITALITY.

A NORMAL active protozoön is a bit of protoplasm in which the vital activities are perfectly balanced, correlated and coördinated in response to internal and external stimuli. If the physiological balance is disturbed by abnormal activity or inactivity in one or other function the result is evident in the general vitality of the organism. The organization, however, is not rigidly fixed and undergoes adaptive changes in response to the new conditions until activities are again coördinated. The Protozoa thus agree with all protoplasm in having the power of adaptation or ability of the protoplasmic substances to react to unusual stimuli in such a way as to maintain perfect correlation and coördination under the new conditions.

An interesting case of orderly response to unusual conditions was the fusion of two conjugating individuals of *Uroleptis mobilis*. Instead of separating at the end of twenty-four to twenty-six hours as in ordinary conjugation, these two individuals remained attached for six days during which time the usual reorganization processes occurred in each. On the seventh day they fused along the entire ventral side, forming a bilaterally symmetrical individual with two oppositely placed mouths and peristomes, two contractile vacuoles and two independent sets of macro- and micronuclei (Fig. 194). On the eighth day this remarkable creature divided three times giving eight double individuals all similar to the original bilaterally symmetrical one from which they came. They continued to divide at the rate of approximately one division per day on the average for a period of four hundred and five days and through three hundred and sixty-seven divisions. The interesting fact here is the correlation of two distinct sets of structures and functions so as to act harmoniously and synchronously as one individual, and the setting up of an entirely new organization. Had the two individuals separated as in normal conjugation their metabolic processes would not have been synchronous, the periods of division would have been more or less similar but not identical. In the double individuals the two sets of eight macronuclei behaved differently in different individuals. In one case each set would fuse prior to division to form a single ellipsoidal macronucleus (Fig. 195) behaving thus like two normal individuals when ready to divide (p. 222). In the

other case the sixteen macronuclei would all fuse to form one single macronucleus which would divide and form two groups of eight each (Fig. 196). In the latter case there was not only a definite adapta-

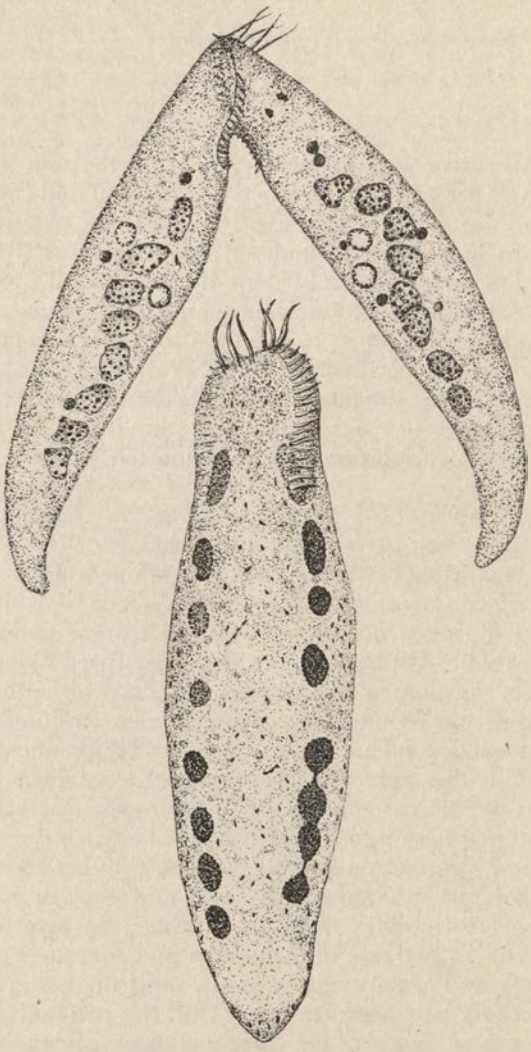

FIG. 194.— *Uroleptus mobilis;* origin of double individual. Above, two conjugating cells; below, the double individual which was formed by the fusion of two such conjugating individuals. (Original.)

tion to the new conditions but a further advance toward a composite animal of a new type and with a novel organization. The synchronous activities indicate that common responses to common

stimuli were operating and that a perfect equilibrium was established throughout.

Vitality, as the sum total of all the protoplasmic activities set up in response to internal and external stimuli is variable. Variations due to external conditions may be readily seen in the effects of heat and cold. Increased temperature increases oxidation leading

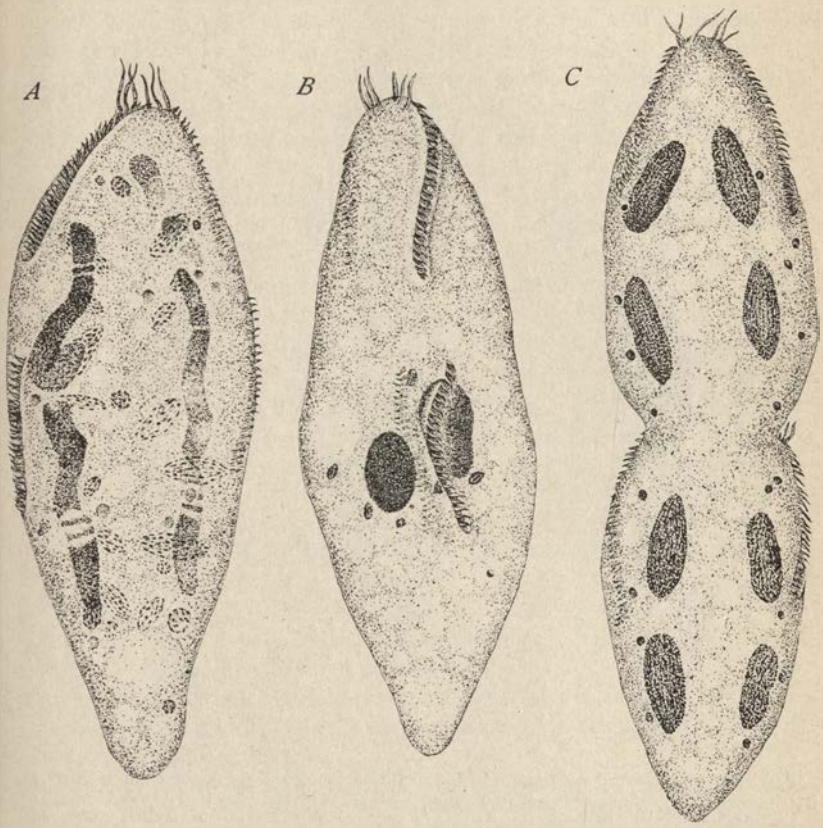

FIG. 195.—*Uroleptus mobilis.* Division of double individual; type with two division nuclei. *A*, stages in the fusion of the two sets of macronuclei independently; *B*, two division nuclei and two new peristomes; *C*, division of the cell each half with two sets of nuclei. (After Calkins.)

to more rapid movements including food-taking activities, more active digestion, assimilation, growth and reproduction. It involves more waste and more active pulsation of the contractile vacuole. Conversely decreased temperature slows up the entire series of activities and vitality is reduced. In like manner any condition of the environment which tends to quicken, to weaken,

or to nullify any one link in the chain of vital activities will have its effect on the general vitality.

It is not improbable that internal reorganization, or disorganization, with increase or decrease of activity in all or in some part of the protoplasmic make-up may bring about similar variations in vitality. Thus changes in organization may be effected by amphimixis or by long-continued metabolic functioning with correspond-

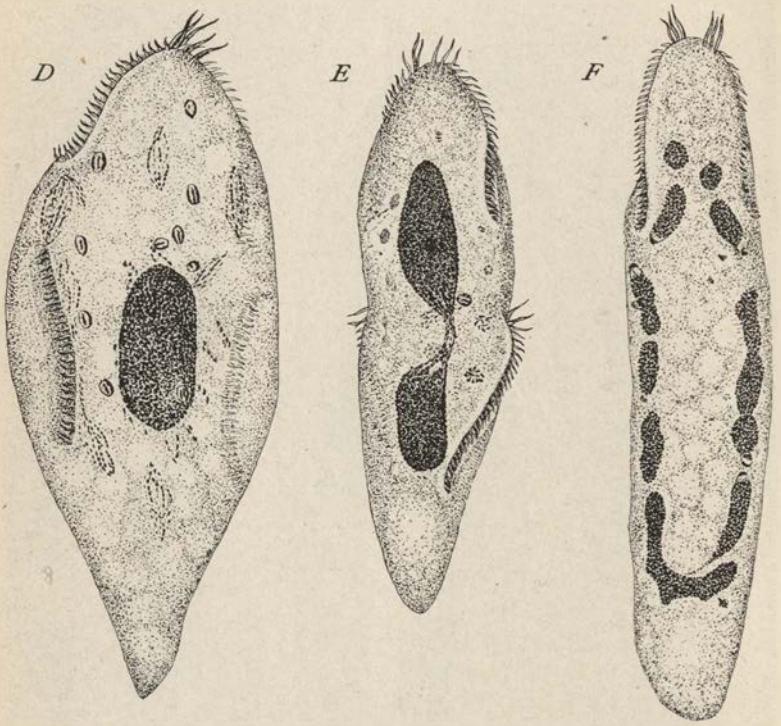

FIG. 196.—*Uroleptus mobilis.* Division of double individual; type with one division nucleus. *D*, the single nucleus formed by fusion of the two independent sets of macronuclei; *E*, first division of the single nucleus; *F*, reconstruction after division with a new type of macronucleus formed from the single division nucleus. (After Calkins.)

ing effects upon the general vitality. The chemical and physical make-up of the protoplasm of an individual may change with continued metabolic activities and lead to a change from what is termed a labile condition when actions, reactions, and interactions are perfectly balanced and at a maximum of activity, to a more stabile condition when these activities become increasingly unbalanced or cease altogether.

I. ISOLATION CULTURES.

The study of protozoön protoplasm by the isolation culture methods has thrown considerable light on these problems of general vitality. If a bit of such protoplasm in the form of a single individual organism, and its progeny by division, is maintained under conditions of food and temperature as constant and uniform as possible, then variations in vitality may be measured and compared in relation to phenomena in the life cycle which are suspected of playing a role in connection with the lability of that protoplasm.

In order to study protoplasm in this manner it is necessary to adopt some measure of vitality which will be an expression of the sum-total of all vital activities. Since every function is a link in the chain of vital activities any one function would do were it possible to measure it accurately, but the difficulty comes with the inability to measure excretion, or nutrition or irritability in any complete and definite manner. Reproduction, however, can be readily measured and being dependent upon the general functions of metabolism, becomes an excellent measure of vitality in a relative and comparative sense. In one way or another the division-rate has been used as a measure of vitality ever since Maupas in 1888 first attacked the problem of age and natural death in Protozoa by the isolation culture method.

In practically any free-living form of Protozoa if proper conditions of food and temperature are provided, the general vitality or sum-total of functional activities as measured by the division-rate, continues more or less uniformly for long periods. The single individuals thus watched appear to be self-sufficient and able to continue their vital activities indefinitely. The question may be raised as it has been raised repeatedly, does the protoplasm of such an individual retain this constant potential of vitality indefinitely, or like a machine, does it wear out sooner or later, and will it ultimately stop altogether?

The problem thus worded is only a partial restatement of the old problem concerning life and death of unicellular organisms which Weismann raised more than forty years ago. He took the ground that Protozoa do not grow old and do not die a natural death, both of which are prevented by an individual dividing into two while in full vigor. The two young ones thus formed by division leave no parental corpse but share the old protoplasm between them and they in turn grow and similarly divide, so that old age is impossible and natural death inconceivable. Weismann further maintained that these fateful phenomena—age and death—are penalties which the Metazoa must pay for their privilege of specialization and differentiation into somatic and germinal protoplasm. Protozoa he compared with the germinal protoplasm of Metazoa in common

with which they have the potential of an indefinitely continued existence.

The experiments of Maupas (1888) to determine by isolation culture experiments whether Infusoria do actually grow old were not convincing. He found, indeed, that a bit of protoplasm in the form of a single infusorian cell if isolated in a suitable culture medium would live, grow and divide. One individual cell formed by such division if similarly isolated, would repeat the process, and from its progeny another representative bit of protoplasm would continue the race. Maupas found that, ultimately, such protoplasm

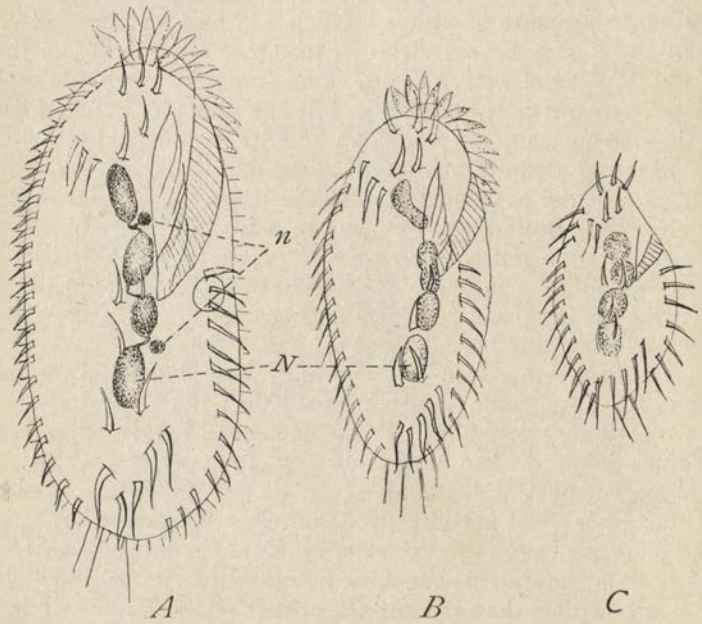

FIG. 197.—*Stylonychia pustulata*, senile degeneration. *B*, *C*, degenerated individuals without micronuclei. (After Maupas.)

would lose its vitality and the race would die after morphological and physiological evidences of degeneration (Fig. 197). In this manner he followed the history of *Stylonychia pustulata* through 316 generations by division when the race died. Another species, *Stylonychia mytilus*, died out after 319 generations; *Leucophrys patula* after approximately 660 generations, etc. The single individual was isolated in culture medium under a cover glass and kept in a moist chamber. Here it divided repeatedly during a period of from two to six days until many individuals were present (in one case 935) all descendants of the original one. One of these was then isolated and the process repeated. From these experiments

he concluded that Infusoria die a natural death after a typical life cycle and after a definite number of generations by division.

The criticism was soon advanced that adverse conditions and bacterial products were responsible for death of his organisms, or, that instead of dying from old age they were slowly killed. There certainly was some justification for this criticism for not only was the covered medium abnormal but the accumulation of bacterial and protozoan products of metabolism might well have been detrimental, particularly if certain types of bacteria gained supremacy. Woodruff (1911) furthermore, has shown that excretion products of *Paramecium* are detrimental to *Paramecium*, and *Stylonychia* products to *Stylonychia*, and the implication is that any type, if continued for long intervals in an unchanged medium will slowly weaken in vitality and ultimately die.

Such criticisms, continued even to the present time in connection with isolation culture work, do not minimize the value of the splendid contribution of Maupas in these pioneer studies on vitality. The present day scepticism in regard to his general conclusion is based upon diverse results obtained by various experimenters with mass cultures as compared with isolation cultures, the great majority of the latter giving results which confirm Maupas. In these the criticism that an unfit environment gradually killed the organisms has been met by the use of carefully prepared culture media and by daily transfers of the experimental organisms to freshly prepared media. In this manner the undue accumulation of bacteria and their products is prevented while the organisms under observation are never present in large numbers.

By use of this method of study the life cycles of many different kinds of ciliates have been established and with the exception of the results obtained by Enriques (1915, 1913, 1916), Chatton (1923) and of Woodruff (1908–1921), they all agree in demonstrating a gradually waning vitality and ultimate death of the protoplasm under observation. The method now generally employed is to start with an ex-conjugant, or individual which has just emerged from conjugation and allow it to reproduce by division three times. Four (Woodruff) or five (Calkins) of the eight resulting individuals are then isolated and continued in daily isolation cultures as "pure lines," four or five pure lines to a "series." For vitality comparisons the daily division rates of all lines of a series are averaged for periods of five days (Woodruff) or ten days (Calkins), and when the cycle is completed the consecutive five or ten day division rates may be plotted to give a graph in which the ordinates represent the average rates of division, the abscissas the consecutive periods. By this method the history of the vitality of the protoplasm under observation is summarized in a graphic and effective manner (Figs. 198, 199, 200).

The above method was first used in connection with the life history of *Paramecium caudatum* (Calkins, 1904), and many other experiments of similar nature were made on this genus by later

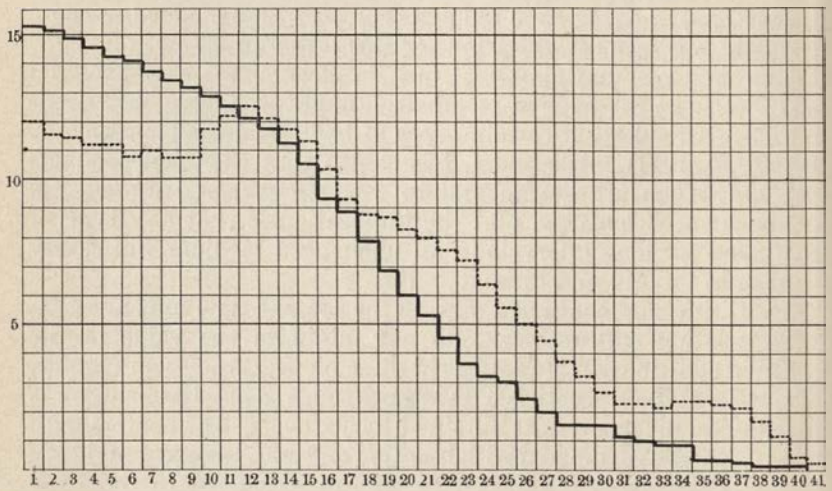

FIG. 198.—Composite graph of vitality of twenty-three series of *Uroleptus mobilis*, each having vitality of more than 85 per cent (solid line). The dotted line is the vitality graph of the double organism. (After Calkins.)

observers. It turned out to be an unfavorable object for the study of this particular problem of vitality, for in 1914 Woodruff and Erdmann announced the discovery of a periodic reorganization process in *Paramecium aurelia* which is exactly comparable with

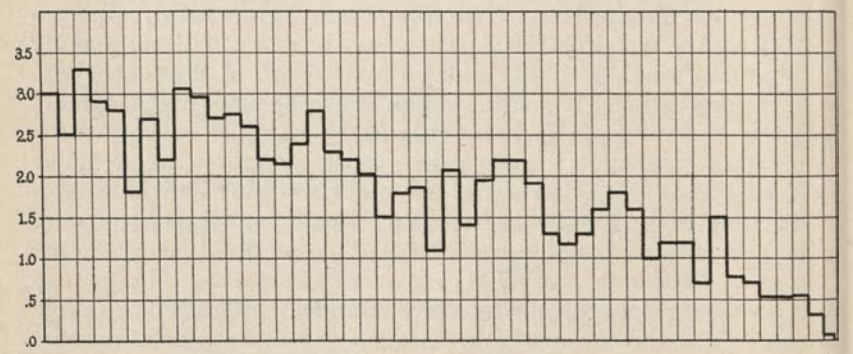

FIG. 199.—Vitality graph of *Pleurotricha lanceolata*. (After Baitsell.)

one type of parthenogenesis occurring in Metazoa (p. 540). The discovery of this reorganization process which they called "endomixis" was the culmination of Woodruff's brilliant and long-contin-

ued study of the life history of *Paramecium aurelia* which he began in 1907, and which had been generally hailed as giving positive proof of the correctness of Weismann's point of view. Parthenogenesis, however, has the same effect upon organization and upon vitality that conjugation has, and as Woodruff and Erdmann showed that "endomixis" occurs approximately once in thirty days in *Paramecium aurelia* and about once in sixty days in *Paramecium caudatum*, any experiments and observations on vitality are valuable only as they lie within these limits of time. For this reason many of the conclusions of Hertwig (1889), of Joukowsky (1898), of Calkins (1903, 1904, 1913) and of Jennings (1909, 1913) drawn from observations on *Paramecium* are of questionable value, and should be used cautiously in connection with the present problem. In other forms however, analogous reorganization processes occur

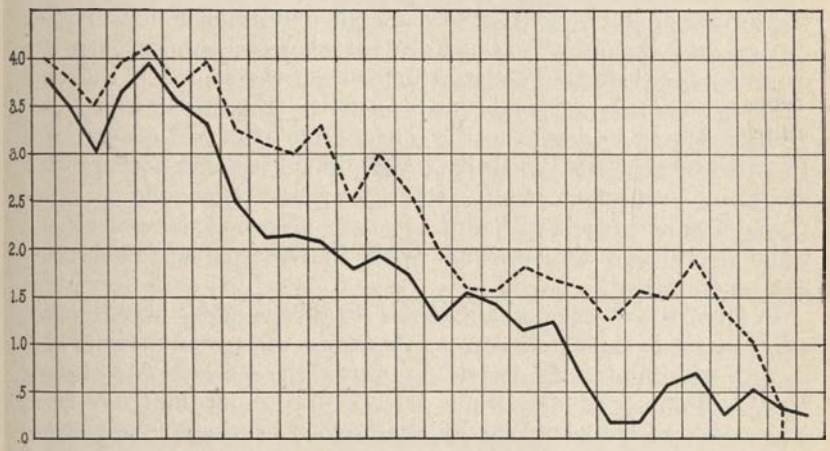

FIG. 200.—Vitality graph of *Spathidium spathula*. (After Woodruff.)

during encystment and are thus advertized in cultures whereas *Paramecium* does not encyst at all but continues under conditions of low vitality to live and move during such periods of depression when "endomixis" is taking place.

While the list of recent experimenters with the Infusoria is rather a long one the actual number of different organisms studied is comparatively small, but different experimenters working with the same species obtained strikingly similar results. Thus *Pleurotricha lanceolata* has been studied by Joukowsky (1898) and by Woodruff (1906), the former following out four series, three of which died out after approximately 220, 250 and 442 generations without conjugation while a fourth was abandoned after 458 generations. Woodruff, using the daily isolation method, found a gradually waning vitality with ultimate death. Baitsell (1914) also carried out isolation

cultures with this organism obtaining a vitality curve similar to that found by Woodruff (see, however, below). *Oxytricha fallax* has been similarly studied by Enriques (1905), by Woodruff (1906) and by Baitsell (1914). The first gives no detailed account of his cultures but makes the general statement that this and other organisms cultivated by him are capable of multiplying asexually *ad infinitum*. Woodruff, however, finds a definite curve of vitality similar to that of *Pleurotricha* with a waning vitality and ultimate death after 860 generations by division, and Baitsell followed the history of three cultures all showing the typical life history, one dying out in the 131st generation, a second in the 159th, a third in the 150th, while a fourth culture in test tubes lived for a longer period but it also finally died, none of these cultures approaching the long history of Woodruff's strain. *Stylonychia pustulata* also has been cultivated by Enriques (1905) and by Baitsell (1912), the former giving no statistical data but maintaining that division can go on indefinitely without degeneration or conjugation if the conditions are right. The latter follows out the history in isolation cultures and finds a typical curve of vitality (Fig. 198) with waning vitality ending in death, in the longest line after 572 generations. In other organisms Woodruff (1905) found waning vitality and death in *Gastrostyla steinii* after 288 generations, and Gregory (1909) a similar result with *Tillina magna* after 548 generations and Calkins (1912) a similar result with *Blepharisma undulans* after 224 generations.

In all of the cases cited above the organisms under investigation are bacteria feeders, and despite the daily change of medium and care in maintaining the isolation cultures the old criticism of bacterial poisoning or deleterious effects of the medium has been repeatedly advanced. Woodruff, however, has kept *Paramecium aurelia* continuously living for seventeen years on the same bacteria diet, "endomixis" occurring at stated intervals and the same observer using the same methods has followed other organisms through periods of waning vitality and death. Metalnikov (1919) similarly has continuously cultivated *Paramecium caudatum* without conjugation. It seems highly probable, therefore, that the prevention of death has little to do with the environment in these experiments but lies in the organisms themselves—with *Paramecium* in the phenomenon of "endomixis."

More direct evidence that bacteria contamination is not responsible for the ultimate death in isolation cultures is afforded by similar experiments with carnivorous ciliates. With these it is possible to use bacteria-free culture media in which the food organisms are introduced with the experimental individual. Again in the majority of cases the ultimate result has been the same as with bacteria eaters. Thus *Actinobolus radians* was followed through

448 generations in isolation cultures in sterile spring water with *Halteria grandinella* as food (Calkins, 1912) and *Spathidium spathula* through 218 generations with *Colpidium colpoda* as food (Moody, 1912) the organisms finally dying in both cases.

Further and very complete evidence that environmental conditions are not responsible for waning vitality and death is afforded by a long-continued study of the protoplasm of *Uroleptus mobilis* an hypotrichous ciliate (Calkins, 1918, 1919, 1920, etc.). This rare organism found and isolated in 1917 is a bacteria eater and was cultivated on a medium consisting of flour and timothy hay boiled in spring water and allowed to stand for twenty-four hours before using. Individuals were transferred daily to such fresh medium in order to avoid an excess of bacteria. For each series of five lines the division rates were figured in ten-day unit periods which were then averaged for sixty-day periods at ten-day intervals. The vitality history of twenty-three series averaged for sixty-day periods, and the history of the double *Uroleptus* are shown in Fig. 198. The average division-rate here for the first sixty days was 15.4 divisions per ten days from which it descended regularly in successive sixty-day periods at ten-day intervals until death. A single series by itself would be no evidence that slow killing had not occurred. But when two of the progeny of a series are allowed to conjugate with one another at any time after the first 75 generations, the ex-conjugants repeat the history of the parent series but do not die when the parent series dies. In this manner the protoplasm of the original *Uroleptus* which was isolated November 17, 1917 is still under observation (June, 1925) although any single series lives from ten months to a year only. The life of the progeny overlaps that of the parent; its progeny overlaps it, etc.; the daily treatment of parents and offspring is identical throughout; both are subject to the same deleterious conditions if present but parents die and offspring live, a history which has been repeated more than 120 times with as many series during the last seven years.

From these clear-cut experimental results with *Uroleptus mobilis* the fact is obvious that under these experimental conditions a fairly uniform life cycle is the rule. The 120 completed life cycles upon which this conclusion is based are all characterized by the same sequence of phenomena, *viz.:* (1) A high initial vitality of the ex-conjugant lasting for a limited period; (2) gradually waning vitality ending in complete exhaustion and death; (3) a period of sexual "immaturity" lasting from the first thirty to ninety days during which encystment may occur if appropriate external conditions are provided but conjugation does not occur; (4) a period of maturity beginning after the first thirty to ninety days approximately and lasting until the ultimate depression when conjugation, under appropriate external conditions does occur; and (5) a period of

old age indicated by morphological degeneration with accumulating physiological depression which ends in death.

The many different series studied furnish ample opportunity for the comparison of vitality in different series. In some there is a greater intensity of vitality, *i. e.*, the average division-rate is higher throughout the cycle; in others the endurance factor is greater, *i. e.*, the individuals live for longer inter-divisional periods without division and the cycle is correspondingly lengthened (see Chapter XII).

On the basis of such consistent experimental results one is tempted to generalize and to hold that all Protozoa pass through a similar life history. The temptation is increased by the confirmation of the main results in connection with an entirely different ciliate, *Spathidium spathula*, in the hands of a no-less competent observer than Woodruff (Woodruff and Spencer, 1924). *Spathidium* is carnivorous and feeds normally on *Colpidium colpoda*. Woodruff and Spencer's isolation cultures were carried on in a basic medium of standardized beef extract to which a few individuals of *Colpidium* were added. The individuals were transferred daily to fresh medium and new food. Many complete series were followed from ex-conjugants, four lines to a series until the protoplasm died a natural death. A typical example is illustrated in Fig. 200, representing the division-rate averaged for five-day periods (solid line) and one offspring series. "The data presented show that in the great majority of cases the cultures died out sooner or later after a somewhat gradual decline in the division-rate" (loc. cit. p. 178). Seventy-nine series ran synchronously with their parent series for at least fifteen days; some of these were then discarded but enough were followed through to afford a justifiable basis for conclusions. Here then we have again a large number of series carried on in isolation cultures, all derived from the same ancestral single ex-conjugant, and dying out "after a somewhat gradual decline in division-rate."

Woodruff, however (loc. cit.), does not grant that the decrease in vitality is due to any intrinsic ageing tendency in the protoplasm but believes that both in *Uroleptus* and in *Spathidium* the proper milieu for continued life has not been provided in the culture methods used, and implies that when a series dies in the absence of conjugation or of endomixis, it is *ipso facto* evidence of a faulty environment. The matter is important for, if Woodruff's conclusion is correct it brings us to an *impasse* in the subject under discussion. He supports his argument with the citation of cases on record in which there is no evident diminution in the division-rate under the conditions of culture, and in such cases he believes that natural environmental conditions have been supplied. He obtained some cases of greater longevity in a few series of *Spathidium*, and although the methods and the culture medium supplied did not differ in any way

from those used for the series that showed decline and death, he concludes that somehow, the conditions were more suitable, and that when suitable the ciliate has the ability or potential for an indefinitely continued existence without the necessity of conjugation (fertilization) or of an equivalent process.

Chatton (1921) shares this scepticism: "One may even conclude" he says, "that the more the facts accumulate, especially those of an experimental nature, the more nebulous does this conception of a life cycle (in ciliates) become" (loc. cit. p. 128). The "facts" thus mentioned include the exceptional results with experimental culture methods by Woodruff as above, by Baitsell, Dawson, Enriques, Mast and others, these being the most prominent, in connection with the Infusoria. As stated above Baitsell (1914) using *Oxytricha fallax* and *Pleurotricha lanceolata* in isolation cultures, obtained results exactly similar to those obtained with *Uroleptus*. For each series the cycle began with high initial vitality which slowly decreased with age until death resulted (Fig. 199). Other series, however, were cultivated in test-tubes and in some cases these continued to live longer than did the individuals of the isolation series. At the time of writing his paper one of these cultures was still alive (*Pleurotricha lanceolata*) six months after the isolation cultures had died. Other mass cultures, although outliving the isolation cultures died (*Oxytricha fallax*). From these results which are not altogether convincing nor consistent, Baitsell concluded that these animals can be bred indefinitely without conjugation or artificial stimulation. The death of all individuals in other mass cultures was attributed to the "cumulative effects of an environment not entirely adapted to the organism." Here again we meet with the idea that if a race dies as it does in the great majority of cases, it is evidence that external conditions are unsuitable. The one race of Baitsell's that continued to live in test-tube mass cultures is obviously an exceptional case and requires explanation. In such mass cultures many things may happen which do not happen in isolation cultures and are easily overlooked. The endurance factor of vitality may preponderate over the intensity factor (in some cases of *Uroleptus mobilis* single individuals lived without division for ninety days). Conjugations may occur and be overlooked where hundreds of individuals are examined in a test-tube, even though such examinations may be made daily and thoroughly. One single ex-conjugant would be sufficient to upset the conclusions drawn from this experiment.

In a similar manner Dawson (1919) found that an amicronucleate race of *Oxytricha hymenostoma* presents a typical cyclical curve of vitality and death follows a gradually decreasing vitality, if the organisms are cultivated in isolation cultures. If maintained in mass cultures they were found to live for a considerable period

longer than the isolated forms, and Dawson concludes that if a suitable medium is provided an indefinite life is possible without conjugation, endomixis or encystment. It is conceivable that environmental media may induce different protoplasmic reactions at different periods of the life cycle, as shown by Gregory's (1925) experiments with *Uroleptus*, and that proper salts in the medium at appropriate periods would enable the protoplasm to maintain its youthful labile condition. Individuals might thus be "doctored" at intervals with a resulting repression of cumulative differentiations and a corresponding maintenance of youth. This was the underlying principle of Woodruff's cultivation of *Paramecium aurelia* on a variable diet, the medium being changed at intervals but in this case without difference in his results. In this connection it is possible that old protoplasm might be reorganized by increasing the permeability and with proper interaction between protoplasm and medium, restored to its original labile condition.

Enriques (1903, 1905, 1916) using *Stylonychia, Oxytricha* and *Glaucoma* found that continued reproduction of these ciliates is possible so long as one cares to follow it provided the "technic is good and bacteria are not too numerous." The physiological deterioration in isolation cultures he attributes to harmful products of bacteria of the medium and maintains, although proof is not given, that neither parthenogenesis nor conjugation is necessary for continued life. His best evidence was obtained with *Glaucoma pyriformis* (1916) which he cultivated for eight months. During this period 2700 generations were recorded, frequently as many as 13 per day and at no time did the organism divide less than 9 times per day (!). It is quite possible as Jennings points out (1920) that endomixis might have occurred without evidence of depression and in the absence of cytological study, was overlooked; or it is also possible that his eight months of observation with the high vitality evidently possessed by this organism, covered only a small part of the entire life history.

With *Didinium nasutum* divergent results have been obtained by Calkins (1913) and Mast (1917). The former found that the division-rate descends from an optimum immediately after encystment to a low minimum just prior to the next encystment and that a fairly uniform number of generations intervenes between encystment periods. The latter gives no data from which the division-rate can be computed but finds a much longer interval between certain encystment periods (conjugation may have occurred), while other intervals agree with those found by Calkins. No evidence was adduced by Mast to indicate cyclical changes in the division-rate but indications of reduced vitality were evident from the statement that "the stock became very weak toward the close—and it is not known how much longer it would have survived." (loc. cit. p. 353).

In other groups than the ciliates, exceptions to the type of life history shown by *Uroleptus* are true of the few cases known. In the animal flagellates for example there is no case of indubitable proof of fertilization in the entire group. On the other hand there have been no successful attempts to cultivate such flagellates by the isolation culture method so that we are entirely uninformed as to the relative vitality in a life cycle. It is possible that processes analogous to endomixis in ciliates take place during encystment stages but as to this we are also ignorant. With these exceptional cases therefore we must wait for further information. With plant flagellates, except for the Phytomonadida, the situation is the same; no complete life cycle has yet appeared and phenomena of encystment have not been studied in detail. In Phytomonadida fertilization processes, often accompanied by sexual differentiation are universal, and some excellent work by Hartmann (1921) with isolation cultures has been carried out. *Eudorina elegans* was followed for upward of five years on artificial culture media and under constant artificial light for part of the time. *Eudorina* is a colonial form of 32 cells embedded in jelly and upon reproduction each of the 32 cells forms a similar 32-cell colony. Each period of reproduction therefore represents 5 divisions of a cell, and in one culture 1500 such cell generations were obtained. Knop's solution, made with distilled water was constantly used and the cultures were maintained free from foreign organisms. Some of Hartmann's earlier series showed evident depression, decline of vitality and death but this result was attributed to faulty conditions.

Hartmann's records are based upon the length of the interdivisional period instead of upon the number of divisions per unit period of time as in pure line work with ciliates. Thus the average interval between generations for the first 10 generations was seven and eighth-tenth days, for the fifth set of 10 generations the interval increased to an average of seventeen and nine-tenth days, etc. His results show clearly the effect of perfected environmental conditions (last 90 generations) and give no indication of periods of depression or of waning vitality. So far as this one series is decisive therefore, there is justification for Hartmann's conclusion that in *Eudorina* division apparently may continue indefinitely in the absence of conjugation or fertilization and without waning vitality.

Exceptional cases are increased through Bělař's observations on *Actinophrys sol* a heliozoön (1924). A single line of his main culture was followed through 1244 generations by division during two years and eight months. Fertilizations were obtained from time to time in mass cultures but these were prevented in the isolation cultures, the latter showing no indication of reduced vitality with continued life (Fig. 201). Bělař also concludes that,

given proper conditions the protoplasm of *Actinophrys* has the possibility of indefinitely continued life and reproduction by division.

In these exceptional cases we meet indeed with diverse experimental results and diverse conclusions. Granted that the experimental work in all cases is done with an equally conscientious regard for controls and pitfalls of all kinds, it is necessary to accept the conclusions on their merits and endeavor to find an explanation which will bring them all into harmony. The first difficulty comes in connection with the popular conception of an abnormal condition of the environment. Except with parasites it is obviously impossible to study the life history of an organism under normal environmental conditions in Nature—in all probability there is no constant "natural" environment. To Enriques, Baitsell, Dawson, Bělař, Chatton, Jollos, and Woodruff in part, the culture methods employed for ciliates are "abnormal" and death is a result of these conditions. With *Uroleptus mobilis* in mind it is difficult to understand by what process of reasoning the conditions of the environment are

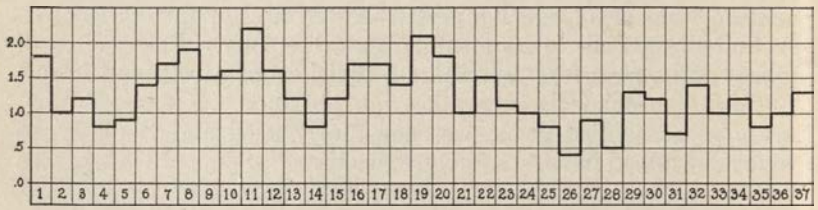

FIG. 201.—Vitality graph of *Actinophrys sol.* (After Belar.)

responsible for the decline of vitality and death when two individuals from such cultural material are restored to full vitality and in the same medium upon conjugation. The conditions are identical for parent protoplasm and offspring protoplasm and yet the former dies, the latter lives until a corresponding age, and dies in turn. The more than one hundred and twenty series that have followed one another since 1917 in the same medium and under the same conditions in the same rhythmical cycles and with surprising uniformity furnish strong evidence that the environmental conditions have been suitable or "normal." For each series there has been the same sequence of physiological conditions—high vitality and sexual immaturity, encystment power, sexual maturity, decline in vigor and ultimate death. If these phases of vitality are normal, if encystment and reorganization, and conjugation, are normal phenomena in the life history of a ciliate then the conditions under which they occur must likewise be normal. A hypercritical mind may deny the existence of conjugation in Nature and maintain that conjugation occurs only under the abnormal conditions intro-

duced when the samples are collected and transferred to small holders in the laboratory. With such an individual convincing proof is apparently impossible and we can only ignore the implication that conjugation is a phenomenon which did not occur under "normal" conditions in Nature but manifested itself only when man began to collect material. I have no sympathy with such a point of view; I regard conjugation as an entirely "normal" process in ciliates as gamete formation and fertilization are "normal" processes in Sporozoa and Sarcodina. When the conditions of the environment are such that this phenomenon does *not* occur, then we may justly look for the unusual at least. The limits of adaptation of protoplasm are unknown to us; it is quite conceivable that conditions may be so arranged that for long periods the normal sequence of phenomena in a life cycle are in abeyance and the impression is gained that protoplasm under such conditions has the possibility of indefinitely continued existence. But can this be considered a normal environment? Here the conditions which lead to conjugation are not offered and such conditions, if any, might reasonably be regarded as abnormal; if conjugation is needed the need is met by the artificial conditions and the organism is more or less adapted to them. No one can maintain consistently that Carrel's long-continued tissue cultures are normal, yet here we have artificial conditions under which these vertebrate tissue cells continue, apparently indefinitely, to live and divide. Death of cells occurs when the transfers are not made at appropriate intervals, but they have become adapted to the artificial conditions of cultivation and continue to live and divide so long as these conditions are maintained.

The question of "normal" or "abnormal" environment after all, appears to me to be of an academic nature, and I cannot agree with Woodruff and his followers in their belief that natural death is not inherent in ciliates under natural, or as he calls it, "normal" conditions. Nor can I accept his further conclusion that the life cycle of a ciliate is a "myth." It is quite evident that the cycle may be greatly varied by reason of external conditions and it is plainly obvious that it has no definite or fixed limits such as postulated by Maupas. If fertilization is an almost universal phenomenon we should be able to determine the conditions which bring it about both within the protoplasm and in the environment. If fertilization satisfies a protoplasmic need we should be able to find out what that need is. When that explanation is forthcoming we shall probably be able to understand why the animal flagellates continue to live so successfully without it.

In regard to the life cycle of Protozoa we are apparently all agreed on some cases. Since the classical work of Schaudinn (1900) on *Eimeria* (*Coccidium*) *schubergi* no one doubts the general

facts of the life cycle in Sporozoa; his work has been confirmed by scores of investigators and upon an enormous number of representative species. A sequence of vital phenomena intervening from fertilization to ultimate gamete formation and fertilization is characteristic of all such cycles and in all cases the race comes to an end with the formation of gametes, and without fertilization the gametes die. Similar cycles are characteristic of Foraminifera and wherever gametes are formed the ultimate fate is the same. With ciliates except in rare instances, gametes are not formed but the organization of the protoplasm undergoes changes at maturity when fertilization processes (conjugation) occur, and in the great majority of pedigreed cultures, the race, like gametes, comes to an end by natural death (see p. 552). The life cycle in all Protozoa signifies the series of events between fertilization and fertilization again or natural death. It involves characteristic changes in organization of the protoplasm and equally characteristic manifestations of vitality. In the following section an attempt is made to correlate these characteristic phenomena in the life cycle with progressive changes in the organization of the protoplasm.

II. ORGANIZATION AND DIFFERENTIATION.

It is evident to any one who has made a study of Protozoa that forms and structures are practically unlimited. It is equally evident that these characteristics are specific for each species. Regeneration experiments show, furthermore, that these specific characteristics are carried in all parts of the protoplasm of an individual, a small part of a *Stentor* becomes a perfect *Stentor*, a small part of a *Uroleptus* develops into a fully differentiated *Uroleptus*, etc. The structure of the adult by which we recognize the species in any particular case is the product of the finer make-up of the protoplasm as it exists in a cyst for example or in a rounded-out fragment cut from the body of an adult. What this finer make-up is is purely conjectural but the idea is carried by the non-committal term "organization" as used in the preceding chapters. In this term we include both the adult structures of the fully formed individual and the undifferentiated protoplasm which has the ability to produce them. There is reason to believe that the differentiations which characterize the adult are brought about as a result of metabolic activities constituting vitality, and these may be induced by changes in environmental conditions as when an organism emerges from a cyst, or regenerates at division periods (p. 484); or they may require a longer period of metabolism and be combined with growth; or they may appear only as a result of cumulative differences representing a gradual change in organization. In general the facts at hand warrant the statement that differentiations

always involve changes in organization and for purposes of description it is convenient to describe them as : (1) Inter-divisional or Ontogenetic Differentiations; (2) Cyclical Differentiations.

1. **Inter-divisional Differentiations.**—In the development of a Metazoön differentiated structures are never present in the initial egg cell but appear in orderly sequence as a result of metabolism, growth and division of cells. A protozoön about to emerge from its cyst is comparable with such an egg cell. The cyst wall becomes permeable, water and oxygen are admitted and metabolism begins. Soon the characteristic motile organs make their appearance differentiated from the apparently homogeneous protoplasm. The oral apparatus, anal aperture, and contractile vacuole appear and the organism emerges apparently complete, from its cyst. This is a rapid differentiation accompanying the onset of metabolism.

Analogous processes of differentiation accompany the regenerations associated with division of the cell. In ciliates a new oral apparatus and specialized motile organs are formed at appropriate positions by the dividing organism (see Chapter V), and differentiation is rapid and complete. The organization under which this differentiation occurs is evidently a result of metabolic activities prior to division (see below).

Differentiations accompanying growth of the cell are characteristic of Protozoa which reproduce by unequal or by multiple division. Here the protoplasm is parcelled out amongst many offspring and each bit of protoplasm, like an encysted cell or a cut-out fragment, possesses the fundamental organization characteristic of the species, but undifferentiated. Thus a bud of *Acanthocystis* or of *Noctiluca* has none of the adult characters but develops them gradually during a period of some days. Or the sporozoite of a polycystid gregarine slowly acquires, with growth, the particular epimerite, protomerite, and deutomerite of its species (Fig. 122). Differentiation occurs here but more slowly than in the case of a ciliate, and is apparently more directly associated with metabolism. Arrested stages in development are not uncommon and frequently lead to puzzling complications in the life cycle. *Trypanosoma lewisi* for example, passes through stages resembling *Leptomonas* and *Crithidia* (Fig. 118) or *Leishmania donovani* through a flagellated *Leptomonas* stage to an adult quiescent intracellular phase. Similarly the young ciliated bud of a Suctorian which may be either parasitic or free-living, gradually loses its cilia develops tentacles and a stalk before it becomes the adult form of the specific description. Again, the young stages of a *Blastodinium* are typically dinoflagellate in character but the embryos develop into the peculiar parenchymatous sac-like adult quite unlike the usual dinoflagellate.

Ontogenetic differentiations combined with growth and cell division are evident in several of the colonial aggregates, particularly in

colonies with definite form and a definite number of cells (*Gonium*, *Eudorina*, *Platydornia*, etc.). Here phenomena are manifested which may well be compared with cleavage and differentiation of the metazoön egg, the resultant cells taking an invariable position in the aggregate. A colony of *Gonium pectorale* for example composed of 16 similar cells, upon reproduction produces 16 colonies. Each of the original cells undergoes a fairly regular cleavage, the cells adhering until a comparatively late stage and finally forming the flattened plate of cells characteristic of the species. The colony *Platydorina* is even more remarkable in the axial relations of the 32 cells composing it (Fig. 3A, p. 21). In *Pleodorina* (*Eudorina*) the cells are differentiated into somatic and germinal, and in *Volvox* the somatic cells form a tissue while the germinal cells are enclosed in the inner jelly, and in the same genus, finally, some colonies form only male gametes and others only female.

The changes in form and structure with growth are to be traced to changes in the protoplasmic organization which in turn are doubtless due to metabolic activities and there is evidence that analogous changes are responsible for the differentiations which accompany regeneration in the more actively developing ciliates. In this connection the merotomy experiments of Calkins (1911) and Young (1922) are suggestive. In Chapter V it is shown that anticipatory changes in the cell precede the nuclear changes. This was first demonstrated by Wallengren(1900) for *Stylonychia* and *Euplotes*, and is clearly shown in *Uronychia transfuga* in which the new posterior giant cirri are formed sometime prior to the nuclear changes in preparation for division. The new cirri appear in a region of the cell previously free from cirri, as well as at the bases of the old cirri. Similarly there is a complete new formation of the peristome with membranelles in the posterior half and a new series of membranelles which replace the old ones in the anterior region. Except for mutilations these regenerations and replacements occur only at periods antecedent to cell division and indicate some far-reaching change in the constitution of the protoplasmic make-up. The ability to undergo such a change furthermore is progressive as shown by experiments in cutting *Uronychia* (Calkins 1911). In these experiments the cell if cut immediately after division in a plane indicated by the section line (Fig. 202), is divided into two fragments one of which, the posterior with giant cirri, contains the single micronucleus, while the anterior portion, with peristome, contains a part of the macronucleus but no micronucleus. In such cases the anterior portion may live for four or five days as an amorphous fragment but it never regenerates the giant cirri. The posterior part however, regenerates the missing anterior region within a few hours and becomes a perfect cell. Exactly the same result invariably follows if an individual is cut when five to eight or

ten hours old after division (Fig. 202). At this time the normal individual is fully grown and active. At the age of sixteen to eighteen hours different results are obtained. If a number of individuals are cut at this age a small percentage of the anterior parts without micronuclei will regenerate into perfect individuals save for absence

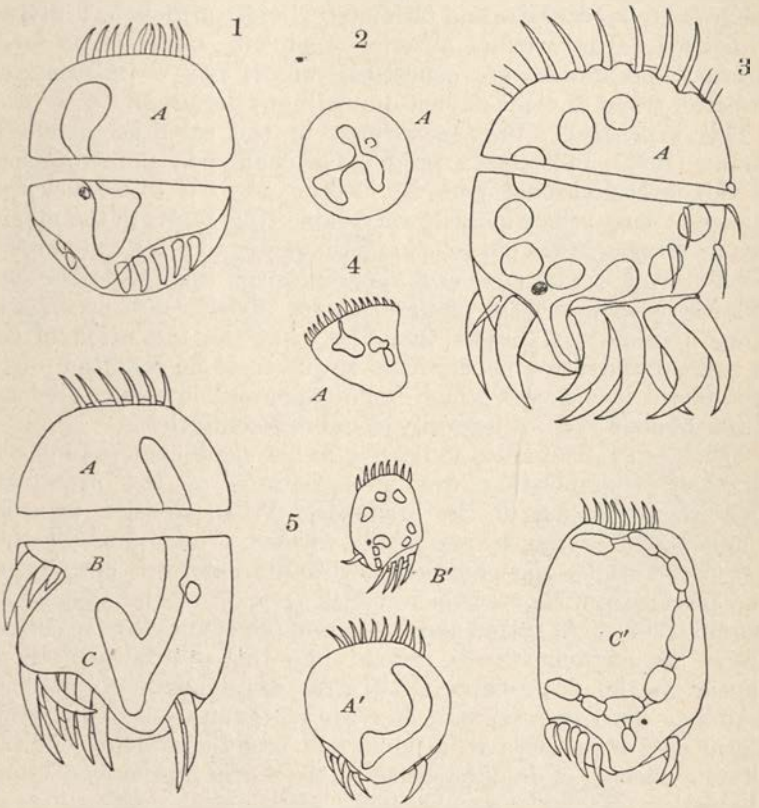

FIG. 202.—*Uronychia transfuga*, merotomy and regeneration. 1, cells immediately after division, cut as indicated; 2, fragment *A* of 1, three days after the operation, no regeneration; 3, cell cut five hours after division; 4, fragment *A* of 3, three days after operation, no regeneration; 5, cell cut at beginning of division as indicated, into fragments *A* and *B*, *C*; *A'*,*B'*,*C'*, fragments *A*, *B*, and *C*, twenty-four hours after the operation; fragment *A* regenerated into a normal but emicronucleate individual *A'*, *B*, *C*, divided in the original division plane forming a normal individual *C'*, and a minute but normal individual *B'*. (After Calkins.)

of the micronuclei; the posterior parts always regenerate. This percentage rises to 100 per cent of cases when individuals twenty-four hours old are cut. Under the conditions at the time the experiments were made divisions occurred in normal animals at intervals of twenty-six hours. Older cells when cut frequently

resulted in the formation of three perfect individuals; one from the transected anterior portion without a micronucleus, and two from the normal division of the posterior portion. One of the latter, the more anterior part, although perfect is of minute size owing to the fact that division of the cell takes place through the original geometrical center, or the "division zone" of the cell. This minute cell grows to normal size and ultimately divides although its division is delayed. The original anterior fragment is perfect as far as external appearances are concerned, but it has no micronucleus and after seven or eight days it dies without dividing.

This experiment, fully confirmed in the essential points by Young (1922), indicates a progressive change in the protoplasm in the inter-divisional period. Except when a micronucleus is present, young cells when cut are unable to regenerate the missing parts. Fragments of old cells have the power to regenerate missing parts even in the absence of a micronucleus. Such regeneration is characteristic of cells in preparation for division and occurs with every division. It follows therefore, that the formation of cirri in these regeneration experiments is due to some condition of the protoplasm in old cells which is not apparent in young ones and illustrates one type of inter-divisional differentiation.

These experiments also indicate another significant phenomenon viz., the reorganization (de-differentiation) of the protoplasm with every division of the organism. When division is nearly completed the power to regenerate without a micronucleus which was possessed by the organism two hours before, is entirely lost and fragments without a micronucleus remain as they were when cut (Fig. 202). As stated above a young cell is unable to regenerate unless the micronucleus is present and this possibility does not appear in the protoplasm until after some hours of metabolic activity. This strongly indicates the reorganization of the protoplasm or a restoration to a labile and undifferentiated condition. Other evidences of de-differentiation are shown by the loss through absorption of the old membranelles, cirri, undulating membranes, oral baskets of the Chlamydodontidæ and kinetic elements of different kinds (see Chapter V) while new elements replacing them are developed from the protoplasm. In this way there is a more or less complete reconstruction or reorganization of the organization at each division.

Another characteristic evidence of inter-divisional differentiation is shown by the polarization of the cell immediately prior to division whereby "division zones" are set up through which division of the cell takes place. Such division zones first described by Popoff (1907) are quite evident morphologically in *Frontonia leucas* and physiologically in *Paramecium caudatum* or *Uronychia transfuga* (Fig. 203). *Paramecium caudatum* when cut near the anterior or

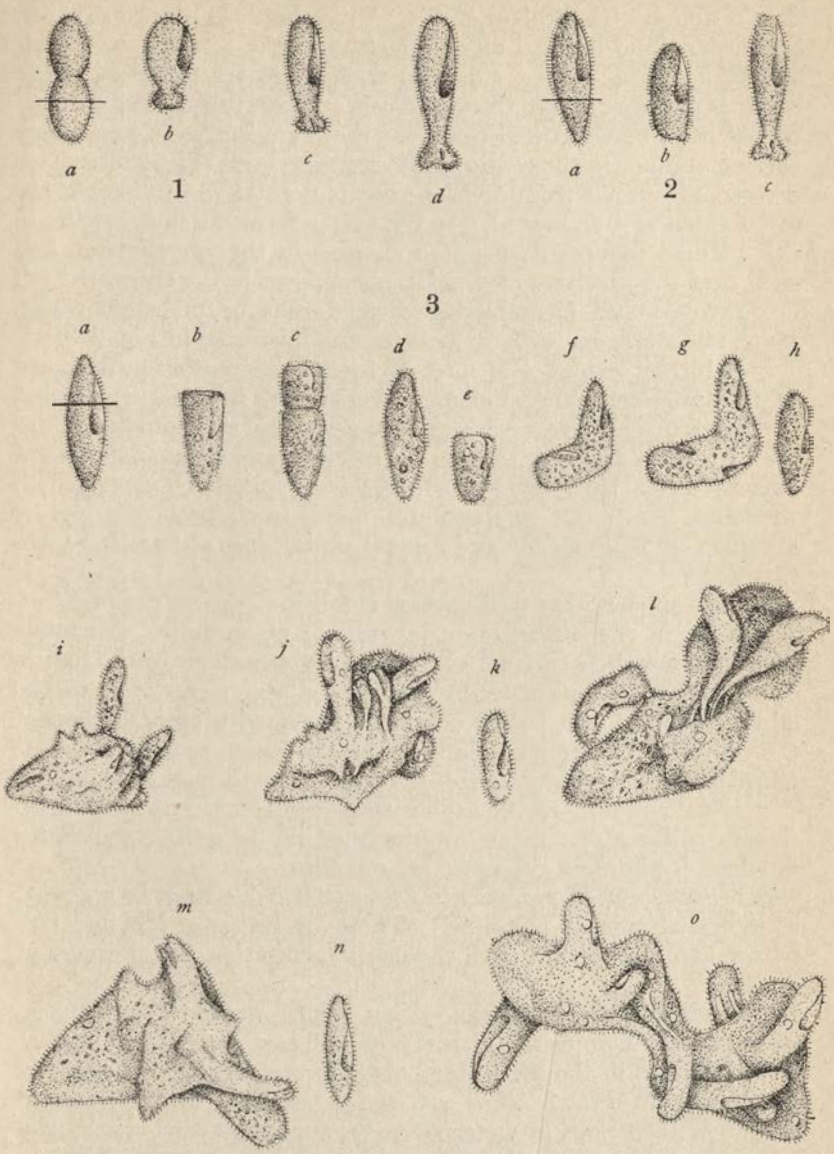

FIG. 203.—*Paramecium caudatum*, merotomy. 1, 2, and 3, different experiments the straight line indicating the plane of cutting; 3, the history of a monster; an original cell 3*a*, was cut as indicated; the posterior fragment (*b*) divided (*c*) into (*d*) and (*e*), the latter formed a monster (3, *f*, to *o*); enucleated individuals (*h*. *k* and *n*) occasionally separated from the parent mass. (After Calkins.)

posterior end as indicated in Fig. 203, does not regenerate the lost part (Calkins 1911, Peebles 1912). A membrane is formed over the cut surface and cortical differentiations in the form of coördinating fibrils, basal bodies, cilia and trichocysts are produced. The result is a characteristic truncated cell. When this divides division occurs in the geometrical center of the organism as it was before cutting and not in the center of the truncated cell (Fig. 203). Two diverse cells result from division; one is normal and full-sized, the other small and truncated. It very often happens that cutting in this manner induces deep-seated changes in the organization and such that the precision of division phenomena in the truncated cell is destroyed and incompletely divided cells or monsters result. (Such monsters, one with 16 mouths, are illustrated in Fig. 203).

Still further evidence of inter-divisional differentiation is shown by the antecedent nuclear changes preparatory to division whereby, in ciliates, macronuclear elements discard part of their substance into the cytoplasm and fuse to form a single, usually ellipsoidal, macronucleus which then divides (*Uronychia, Stentor, Uroleptus, Spirostomum*, etc.). Or in flagellates the entire kinetic complex is absorbed in *Lophomonas* and several other types of flagellates (see Chapter V).

It thus appears that well-marked changes of the nature of differentiations in the organization are taking place during the inter-divisional metabolic period, and that transformations of the nature of de-differentiations whereby the protoplasm is restored to the labile condition of a young organism, occur with each division of the cell. It is quite possible that this divisional reorganization is adequate for the preservation of the protoplasm through long periods of activity and may be the explanation of the long-continued life in certain cultures of ciliates, or continued life of animal flagellates in which fertilization processes are unknown.

Other differentiations occur in Protozoa which cannot be regarded as inter-divisional in character. These are rather of a cumulative nature and are not lost with the de-differentiation which occurs at division.

2. **Cyclical Differentiations.**—This second group of differentiations are not manifested in every cell of a species but appear at certain phases in the life history of the protoplasm composing any series of individuals. They are racial therefore and correspond roughly with periods in metazoön development such as youth, adolescence and age. Some of these differentiations are characteristic of very young forms, occurring immediately after fertilization and at no other time in the life cycle. Others make their appearance later in the cycle and often after many generations by division. These lead to and accompany the phenomena of fertilization and include gamete formation and maturation stages. Still others

occur only at the end phases of the life cycle and are specific characteristics of age. We find justification therefore, for purposes of description at least, in presenting facts concerning differentiations of youth, of maturity and of age, but we have no intention of setting limits to these phases or of assuming that an ageing process is inherent in Protozoa.

A. Cyclical Differentiations Peculiar to Youth.—Intensity of metabolic activities is one of the characteristic features of young organisms but with Protozoa exact data are difficult to get except from isolation cultures. In such cultures intensity is indicated by the division-rate and the great majority of ciliates show a higher division-rate in the early periods of vitality (see p. 558 and Figs. 198 to 200). In *Uroleptus mobilis* this intensity lasts for approximately sixty days (Fig. 198) and in *Spathidium spathula* for about forty days (Fig. 200). The evidence is not consistent however if all isolation cultures are considered and in exceptional cases of *Uroleptus* and of *Spathidium* there is no indication of this relative intensity. Nor does Bělař give any evidence of it in his isolation cultures of *Actinophrys sol;* nor does Hartmann (1921) for *Eudorina elegans.* With parasitic forms exact data in this matter are wanting and general impressions are of little value.

Young organisms show the effects of abnormal conditions of the environment more quickly and more intensely than do older ones. Gregory (1925) for example has shown that salts and change of medium are deleterious to very young forms of *Uroleptus mobilis* while older forms are not affected. This is in line with Child's results in connection with the action of potassium cyanide on many kinds of organisms, those parts which have the highest metabolic rate being first to succumb.

The differentiations indicated above are physiological in nature and are rather intangible. Other differentiations characteristic of youth while also physiological are indicated by morphological or structural modifications. Of these the most noteworthy are the different types of cysts which are secreted by all kinds of Protozoa. Encystment has been generally regarded as a means of protection for the organism against adverse conditions of the environment. This is probably more traditional than accurate, for very few Protozoa are actually known to encyst when the external conditions are unfavorable. Mast (1923) for example finds that food and temperature have little effect in causing *Didinium nasutum* to encyst but encystment takes place under the best conditions. It is more probable that organisms which have had the power to encyst persist under such conditions while the great majority are killed. Cutler (1919) however gives evidence to show that skatol induces encystment in *Endamœba dysenteriæ.* This power to encyst appears to be a factor of young organisms induced

possibly, as Mast (1923) suggests, by the accumulation of waste materials. In a relatively few cases, however, Protozoa will encyst as a preliminary to reproduction by division (*Tillina magna, Colpoda,* etc. among the ciliates, and in some flagellates).

The sporoblast capsules of all Sporozoa with the exception of the Cnidosporidia (p. 451), are formed as a result of the first activities of the young fertilized cell and they do not occur again. The same phenomenon is characteristic of zygotes in Sarcodina and Mastigophora. With Infusoria where fertilization is accomplished through conjugation such zygote cysts are practically unknown, but encystment, with reorganization processes, is possible during the early period of the life cycle until maturity, when it is apparently replaced by conjugation. Thus in *Uroleptus mobilis* in connection with which this phenomenon has been carefully studied, encystment may occur within three days after fertilization but usually after a longer period has elapsed. Such encystments occur under the same external conditions as do conjugations later in the cycle. So-called "conjugation tests" are made every week or ten days. For these, all of the individual cells of a series left over after a daily isolation has been made are placed in a large container with fresh medium. Here they are allowed to accumulate until thousands of individuals are present. The food medium is not replenished and such mass cultures are watched daily until the individuals die. After five or six days conjugations will take place provided the organisms are mature; if they are not mature encystment takes place and it frequently happens that thousands of cysts are present in one container. From the records made during the last seven years it is possible to work out the incidence of encystment and of conjugation for each series. Fig. 204 shows the vitality curve of ten different series. The periods of the first encystments observed and the last encystments in the different series are connected by vertical lines. The first appearance of conjugation is indicated in the same manner but with double lines. In some series it happens that both encystments and conjugations occur in the same container but tests of the same series made later give only conjugations. With *Uroleptus* at least it appears therefore that encystment is a characteristic phenomenon of young organisms comparable with the Dauersporen of phytoflagellates, and lower plants generally, after fertilization; and that the power to encyst disappears with the advent of maturity. It is highly desirable to have similar data for other types of ciliates; some incomplete studies on *Didinium nasutum* indicate analogous phenomena (Calkins (1915).

B. Cyclical Differentiations Peculiar to Old Age.—Toward the end of the life cycle even more characteristic differentiations occur than at the outset. In many cases these are coincident with the fertilization phenomena and will be discussed in connection with

differentiations at maturity. The most significant of these age differentiations are: (1) Greatly reduced vitality; (2) structural differentiations; (3) abnormal divisions leading to monster formation; (4) special structures appearing at no other time in the life cycle.

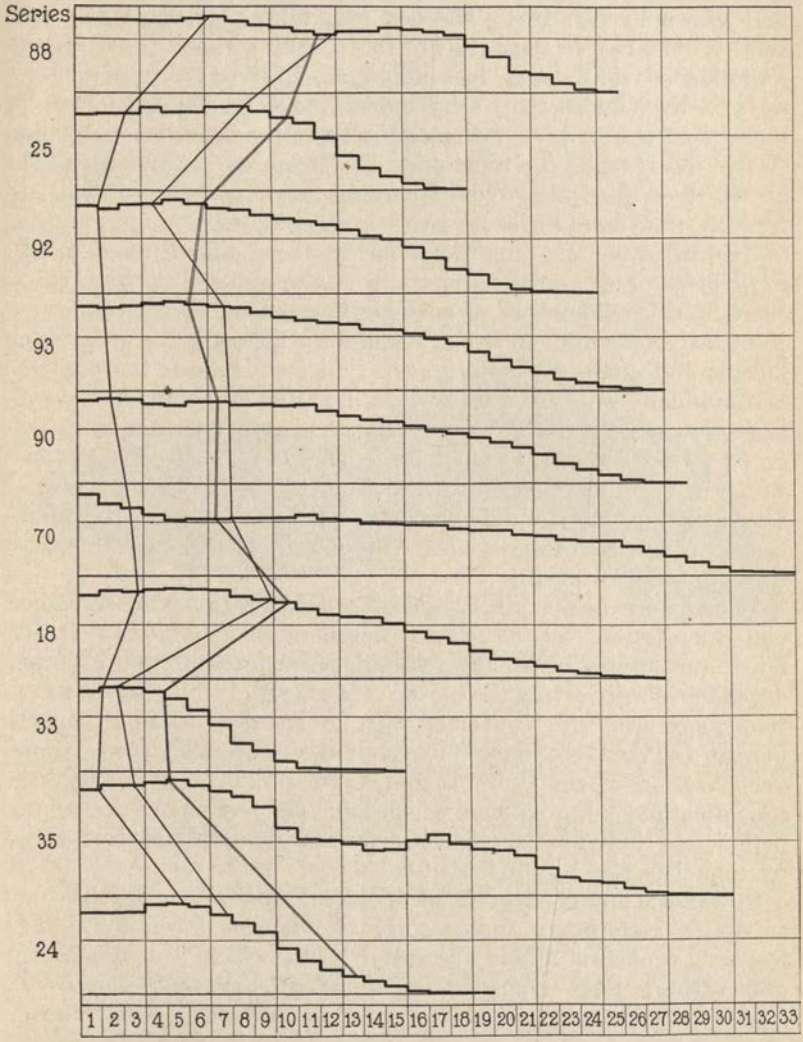

FIG. 204.—Vitality graphs showing the limited period of encystment (between the two irregular vertical single lines), and the periods at which conjugation begins (double line) in ten different series of *Uroleptus mobilis*. (Original.)

The best evidence of reduced vitality towards the end of the cycle is afforded by *Uroleptus mobilis* and *Spathidium spathula*. In

the former, series after series have been followed from high initial vitality after fertilization until death occurred. In more than one hundred and twenty such series the history has been the same but with variations in time and in number of generations well illustrated by the series selected from the records for different years and shown in Fig. 198. The last individuals of such series may show a remarkable tenacity in vitality but without the power to reproduce. Of 283 such "last individuals" 1 lived more than ninety days; 2 lived more than sixty days; 7 more than forty days; 15 more than thirty days; 26 more than twenty days; 88 more than twelve days; while the remainder lived from one to ten days. In all of these cases the old individuals were transferred daily to fresh medium from the same source as that in which other, younger, individuals were dividing from one to three times per day. In most of the old specimens apart from the reduced division rates, there is little evidence of physiological weakness. They move with the usual vigor and apparently maintain an equilibrium between income and outgo for many days. This condition is the outcome of a gradually waning vitality which in turn may be due to a slowly increasing stability of substances in the protoplasmic organization, or as Robertson (1921) suggests, to accumulation of substances which can no longer be discharged from the cell. This I interpret as evidence of old age differentiation with the same fatal termination as that which follows highly differentiated gametes which fail to unite in fertilization.

In many organisms this physiological deterioration is accompanied and manifested by structural degenerations. Maupas (1888) noted the loss of micronuclei in old age ciliates as well as other degenerations involving the motile organs (Fig. 197). The observations have been fully confirmed with *Uroleptus mobilis* particularly in regard to the loss of micronuclei, but also noticeable in the extreme vacuolization of the protoplasm (Fig. 7, p. 28). In *Paramecium caudatum* and in individuals which have not conjugated for a long period, old individuals are characterized by hypertrophy of the micronucleus and by the loss of trichocysts in the cortex.

Still another outcome of the physiological weakness is the tendency to divide abnormally thus leading to monster formation. This has been typical of all old age cultures which have come under my observation. Such monsters are strikingly like those formed as a result of cutting *Paramecium* (see supra p. 486) but they never grow into large amorphous masses of protoplasm which frequently develop from mutilated Paramecium individuals (Fig. 205).

The old age phenomena discussed above all involve a physiological weakness or reduced vitality which may well be traced back to increasing stability of protoplasmic substances, and lead to a break-down in the protoplasmic organization. A fourth type has

to do with protoplasmic differentiations of a formative character and involves structures which appear for the first time, and only, when the protoplasm is old, probably as a result of the cumulative differentiation which has taken place. The sporoducts of the gregarines furnish a good illustration of this phenomenon. Here in *Gregarina cuneata*, accordingly to Kuschakewitsch (1907) the old nucleus gives rise to a minute germinal nucleus while the remainder is distributed as chromidia throughout the cell. The characteristic sporoducts grow into the brood cavity of the sporocyst in the form of tubules at the bases of which the observer found collections of chromidia (Fig. 121, p. 244). Similar observations have been made upon other sporoduct-bearing forms (*Clepsidrina*, *Gregarina ovata*, etc.). These are final products of protoplasmic activity with the prospective function of sporoblast elimination and have nothing at all to do with fertilization (see Chapter XI). Also in the Cnidosporidia some of the residual nuclei and protoplasm become differ-

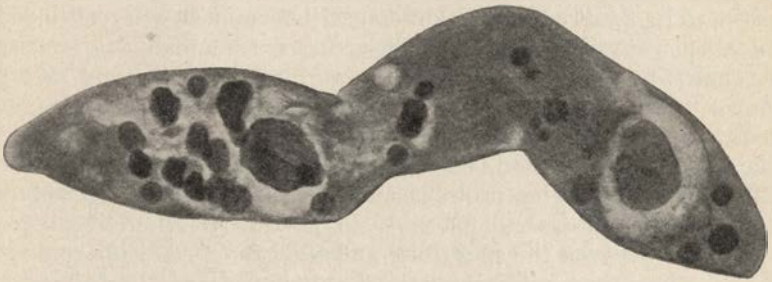

FIG. 205.—*Paramecium caudatum* monster, a type common at periods of old age. (After Calkins.)

entiated into sporoblast capsules while others give rise to the peculiar polar capsules and the threads characteristic of these Sporozoa (p. 446).

In a number of Sarcodina, as in Gregarinida, there are special morphological structures for the purpose of distributing the mature products of multiple division. These are frequently quite complex, the elaters and capillitia of Mycetozoa for example, recalling the spore-disseminating elements of the higher plants. The life history is varied, the complications being due mainly to the formation of multinucleated plasmodia by fusion of numerous multinucleated cells and to fruiting or spore structures which arise from the plasmodium. According to the later observations of Jahn (1911) the plasmodium begins as a single zygote in the form of an amœboid cell with one nucleus. This nucleus divides repeatedly resulting in a multinucleated cell and plasmodia are formed by fusion of such cells. When mature the plasmodium gives rise to

the elaters through the activity of nuclei which degenerate with the process. In some forms the old plasmodium loses water, dries and forms a hard indurated crust called a sclerotium. In the majority of forms the protoplasm becomes transformed into a tough skin or membrane termed the peridium which may be strengthened by deposits of lime. Other parts of the protoplasm become modified into felted spore capsules or capillitia through which the elaters ramify.

In all of these cases of old age protoplasm the evidence justifies the conclusion that the organization has become profoundly changed, the change often resulting in useful morphological and physiological differentiations. The changes are of a character however which prevents any recovery of vitality and death of the protoplasm results unless gamete formation and fertilization supervene.

C. Cyclical Differentiations Peculiar to Maturity.—Sexual maturity in Protozoa is not a theory but a fact demonstrated in many different kinds of Protozoa. In many cases the young form slowly grows to its adult condition; differentiations appear with continued metabolism until the individual becomes a gamont and gives rise to gametes. Thus in polycystid gregarines the sporozoite slowly grows to its definitive size and differentiations appear with that growth. The protoplasmic conditions leading to gamete formation may, with equal reason, be regarded as evidence of still further differentiation in the protoplasmic organization. In Schizogregarinida and in Coccidiomorpha an asexual reproductive cycle intervenes between the sporozoite and the gamont and the same is true in the Foraminifera and the Phytomonadida. In Infusoria, as Maupas long since demonstrated, fertilization is possible only after a period of vegetative metabolism and reproduction. Sexual maturity in general therefore, like other conditions of protoplasm, may well be interpreted as evidence of specific differentiations of the protoplasmic organization.

Few problems in biology have attracted more attention than those associated with sex, and attempts to interpret the phenomenon have been as varied as they are sometimes ingenuous. The very definition varies with different interpreters, the usual definition involving association of the concept sex with peculiarities of structure and function which enable an observer to distinguish males from females. Others regard sex as evidence of a fundamental difference in protoplasm, one type giving rise to males, another type to females as in Weininger's arrhenoplasm (male producing) and thelyplasm (female producing). Or the differences of sex according to Minot (1882) and Schaudinn (1904) are due to specific types of chromatin both of which are present in all individuals derived from a fertilized cell, but male chromatin predominating in males, female chromatin in females. Still others

interpret sex differences as originating through metabolic activities, segregation of protoplasm thus differentiated, and distribution by inequalities in division of the cell as Bütschli first suggested.

Not only somatic differentiations with their specific functions, but products of such differentiation in the form of gametes together with the causes which bring about the attraction and fusion of gametes, are all bound up in the ultimate significance of sex. Somatic differentiations indicating male or female types are extremely rare in Protozoa but problems of gamete formation and fusion are presented by Protozoa of all kinds and so far as it applies to such problems, the term sex and its connotations apply to the unicellular animals.

There is little reason to doubt that a fundamental effect of sex is the perpetuation of the species through union of gametes; and there is equally little reason to doubt that the same function underlies conjugation and fertilization generally in Protozoa. It is tacitly understood by biologists that the sum-total of conditions leading to the production of eggs or of spermatozoa is typical of the female or of the male, hence egg-like gametes in Protozoa are regarded as the result of female activities, while spermatozoa-like gametes come from males. This line of thought has led to the widespread custom of describing macrogametes in Protozoa as female and microgametes as male organisms. A difficulty has arisen however, in connection with the entire absence of visible differences between the gametes of many species distributed amongst all groups of Protozoa, and here obviously, the attempt to apply any definition of sex fails completely. Yet such fertilizations are as fruitful and as important for the species as are those in which gametic differences are well-marked.

There are two fundamental biological problems associated with the formation and fusion of gametes. These are: (1) The explanation of the origin of gametic differences, and (2) explanation of the phenomenon of attraction of gametes followed by their temporary or permanent fusion. It would be mere presumption to claim that our present state of knowledge permits an explanation of these phenomena, but there is an abundance of data from which working hypotheses may be deduced.

Gametic Differences.—In Metazoa differences in gametes are reduced to practically those between egg and spermatozoön. In Protozoa there is no common type of difference but all gradations may be found here from apparently similar individuals to differentiated eggs and spermatozoa. This has led to attempts to classify gametes for purposes of description, into those which are similar (isogametes) and those which are dissimilar (anisogametes). Similar gametes, however, may be minute derivatives of adult individuals —microgametes—or they may be adult individuals which cannot

be distinguished from ordinary asexual, vegetative individuals. The latter type is represented by the vast majority of Infusoria, and, as Minchin maintained, there is very little justification for calling them gametes at all; yet they come together for purposes of

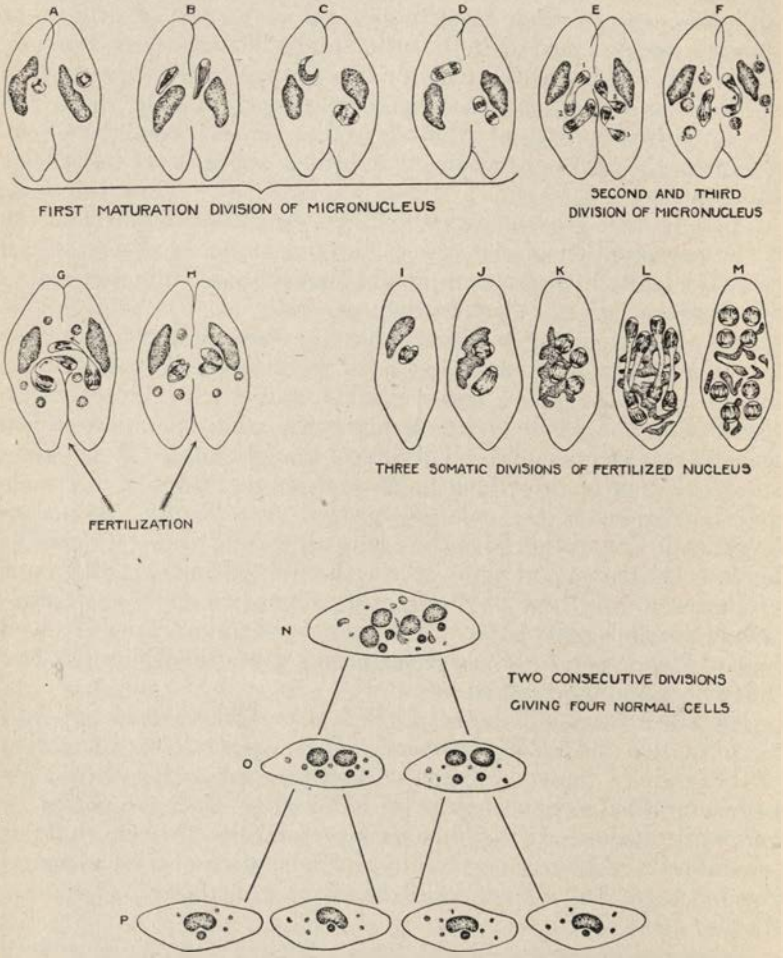

FIG. 206.—*Paramecium caudatum*. Diagram of the fertilization processes. (After Calkins.)

fertilization and to this extent at least, resemble gametes. In the majority of Protozoa fertilization involves the permanent fusion of cell bodies as well as of cell nuclei and the term copulation is applied to such cases. In the Infusoria fertilization involves the permanent fusion of nuclei only, while the cell bodies, with few exceptions, are

incompletely fused and this is only temporary (Fig. 206). To this phenomenon the term conjugation is given. A conjugating ciliate, however, is physiologically different from a vegetative individual and may be distinguished by the general designation gamont. These considerations lead to the following classification:

(*a*) *Conjugation.*—Temporary cell fusion of gamonts; permanent nuclear fusion.

(*b*) *Copulation.*—Permanent fusion of cell bodies and cell nuclei of gametes.

Gametes
- *A.* Isogametes
  - (*a*) Similar macrogametes or gamonts (hologametes).
  - (*b*) Similar microgametes.
- *B.* Anisogametes
  - (*a*) Dissimilar microgametes.
  - (*b*) Macrogametes and microgametes (oögametes).

(*a*) *Hologametes and Conjugants.*—The nearest approach to conjugation of the ciliates is to be found in the fertilization phenomena (pseudo-conjugation) of the Sporozoa, particularly in the Gregarinida. Here, two gamonts (gametocytes) come together but do not fuse; after the formation of a common cyst each cell proceeds to form a number of gametes which may be isogamous or anisogamous. After the gametes are formed the gametocytes degenerate and disappear while the gametes fuse two by two in copulation. In the coccidian *Adelea* the phenomena are more nearly like those of the ciliates. Here a microgametocyte and a macrogamete become associated in conjugation and without the formation of a cyst membrane. The former produces four or more nuclei by division and one of these penetrates the macrogamete and fuses with its nucleus. One of the conjugants thus resembles a ciliate while the other one, the microgametocyte, resembles a gregarine in that it degenerates and disappears. In ciliates there is a mutual formation of gametic nuclei, a mutual interchange, and a mutual fertilization. Here both individuals correspond to the macrogamete of *Adelea*.

It is possible that the peculiar conditions existing in present-day ciliates may have resulted from conditions of pseudo-conjugation as illustrated by the present-day gregarines, and that originally, a group of gametes were formed which united to form zygotes outside of the parent cells, or inside as in the case of *Ophryocystis mesnili** (Fig. 180, p. 425). On this hypothesis which has been

* Some of the parasitic ciliates suggest the gregarines in their conjugation phenomena. Thus in *Balantidium coli*, according to Brumpt (1909), two individuals come together and form a common enveloping cyst membrane within which the two cells now completely fuse.

very generally accepted by protozoölogists, the fusing nuclei of conjugating ciliates are interpreted as the nuclei without cell bodies of gametes, such as those of *Ophryocystis*. An interesting observation by Dogiel (1923) on the parasitic ciliate *Cycloposthium bipalmatum* and in other Ophryoscolecidæ as well (Dogiel 1925) lends some support to this theory. Here gametic nuclei are formed as in other ciliates; one of these nuclei, the migrating nucleus, develops a tail and, like a spermatozoön, makes its way through the membrane of the peristomial region of the mother-cell, and into the external chamber formed by the mode of fusion of the two gamonts (Fig. 207). From this chamber it enters the other gamont by way of the mouth and ultimately meets and fuses with the stationary nucleus of this gamont.

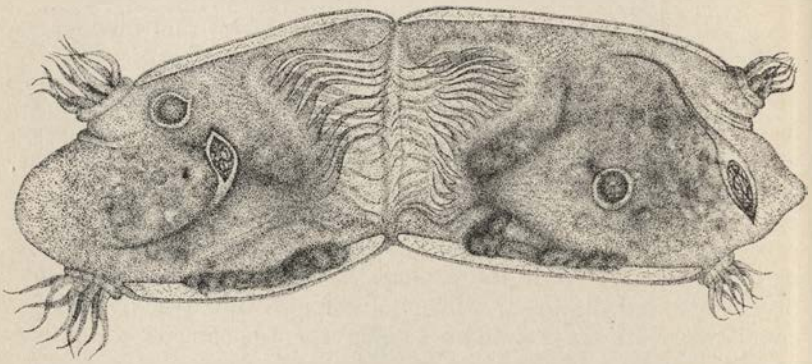

FIG. 207.—*Cycloposthium bipalmatum*. Conjugating individuals with spermatozoön-like wandering nucleus. (After Dogiel.)

(*b*) *Isogametes and Anisogametes*.—The term copulation as used in connection with the Protozoa refers to total and permanent fusion of gametes. Of these there is the greatest variety of structures and differences in different types of Protozoa. In very few cases of isogametes do we find copulation between individuals whose differentiations are not expressed by morphological characteristics. In such types the individuals differ little if at all from the ordinary vegetative forms except in a physiological sense. Plastogamy or casual cell fusion is easily mistaken for such hologamic copulation and descriptions of so-called fertilization processes in *Noctiluca*, in testate and in naked rhizopods, in Heliozoa and in different types of flagellates are open to criticism on this ground. In the case of *Scytomonas* (*Copromonas*) *subtilis* (Dobell 1908) and *S. minor* (Berliner 1919) the evidence appears to be fairly convincing that copulation of hologametes actually does occur, but even in these cases, the interpretation is not above criticism (Fig. 208).

The majority of isogametes show morphological characteristics which easily distinguish them from agametes or vegetative individuals. In many cases the physiological differences at maturity are expressed by a change in the type of division whereby binary fission is replaced by multiple division. Many daughter cells are thus formed from one gametocyte and the term merogametes has been applied to such a brood. The copulating gametes, however, show no distinguishing morphological characteristics and the differences between them if there are any, must be of a physiological nature. In Foraminifera such isogametes are the rule and their

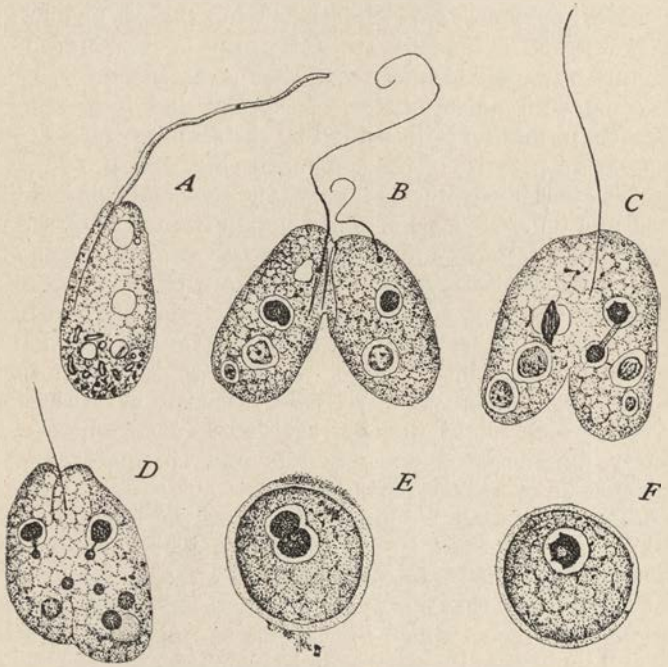

FIG. 208.—*Scytomonas subtilis*, copulation. (After Dobell.)

formation indicates a well-defined cyclical differentiation of the parental protoplasm. Thus in *Polystomella crispa* according to Schaudinn (1903) and Lister (1905); in *Peneroplis pertusus* according to Winter (1907); in *Trichosphærium sieboldi* according to Schaudinn (1899) and in Foraminifera generally, the young protoplasm after fertilization forms one type of organism termed the microsphæric generation which reproduces by agamete formation (Fig. 119, p. 239). Such agametes develop without fertilization into organisms of a different type, the difference being shown by the character of the initial shell chamber, hence a macrosphæric generation. After

metabolic activities and full growth the macrosphæric organism breaks down into a multitude of isogametes which have an entirely different organization from that of the agametes. Whereas the latter are pseudopodiospores, the isogametes are flagellispores, each bearing two similar flagella, and copulation occurs by union of two of these similar flagellispores (Fig. 119, *A*, *C*).

Isogamous microgametes are also quite common in Phytomonadida and in their formation the usual type of binary fission gives place to multiple division whereby from four (*e. g.*, *Polytoma uvella*) to many (*e. g.*, *Stephanosphæra*, *Chlamydomonas*) isogametes are formed. Also in the Sporozoa, particularly amongst the Gregarinida, isogametes are characteristic but there is no uniformity in the group and a tendency to anisogamy is pronounced. The same genus (*e. g.*, *Monocystis*) comprises some species with isogametes, some with anisogametes. A special and interesting case of isogamete formation is illustrated by the schizogregarine *Ophryocystis mesnili* a parasite of the cockroach (Fig. 180, p. 425).

Anisogametes illustrate not only the cyclical differentiation resulting in a different type of reproduction from that of the usual vegetative type but they also illustrate the two divergent effects which such differentiations may bring about, one leading to relatively greater stability, storage of metabolic products and relative inactivity, the other leading to a more kinetic organization with freedom from metabolic products. As one would expect there is every gradation in the relative differentiation of anisogametes from forms which might well be regarded as hologametes to the completely differentiated egg-like cells and spermatozoa of the Coccidiomorpha or Volvocidæ.

According to Schaudinn's interpretation of the fertilization processes in *Actinophrys sol* (1896) there is a permanent fusion of similar adult cells (hologametes). But the recent investigations of Bělař (1922) show that one of the apparent hologametes develops a pseudopodial process which is the first to unite with the other gamete and undergoes its meiotic divisions more quickly than does its mate (Fig. 209). Similar minute differences in microgametes are characteristic of *Monocystis rostrata* but the differences become more pronounced in *Pterocephalus nobilis*, *Schaudinella henleæ*, or *Stylorhynchus longicollis*. In Phytomonadida also, slight differences may be facultative in *Polytoma uvella*, more pronounced in *Chlorogonium euchlorum* and extreme in the genus *Volvox* (Fig. 211, p. 504). In Sarcodina, apart from *Actinophrys sol*, there are few cases in which the full development and fusion of anisogametes have been convincingly demonstrated. Schaudinn (1903) described the formation and union of anisogametes in a *Centropyxis aculeata* but the confirmation of his arcelliform gametes has not yet appeared. Elpatiewsky (1909) described the fusion of anisogametes in *Arcella*

vulgaris as a part of a very complex life cycle. In both of these testate rhizopods the nuclei of the gametes are derived from chromidia formed in the gametocytes while the cell bodies are formed by multiple division of the protoplasm. In Radiolaria according

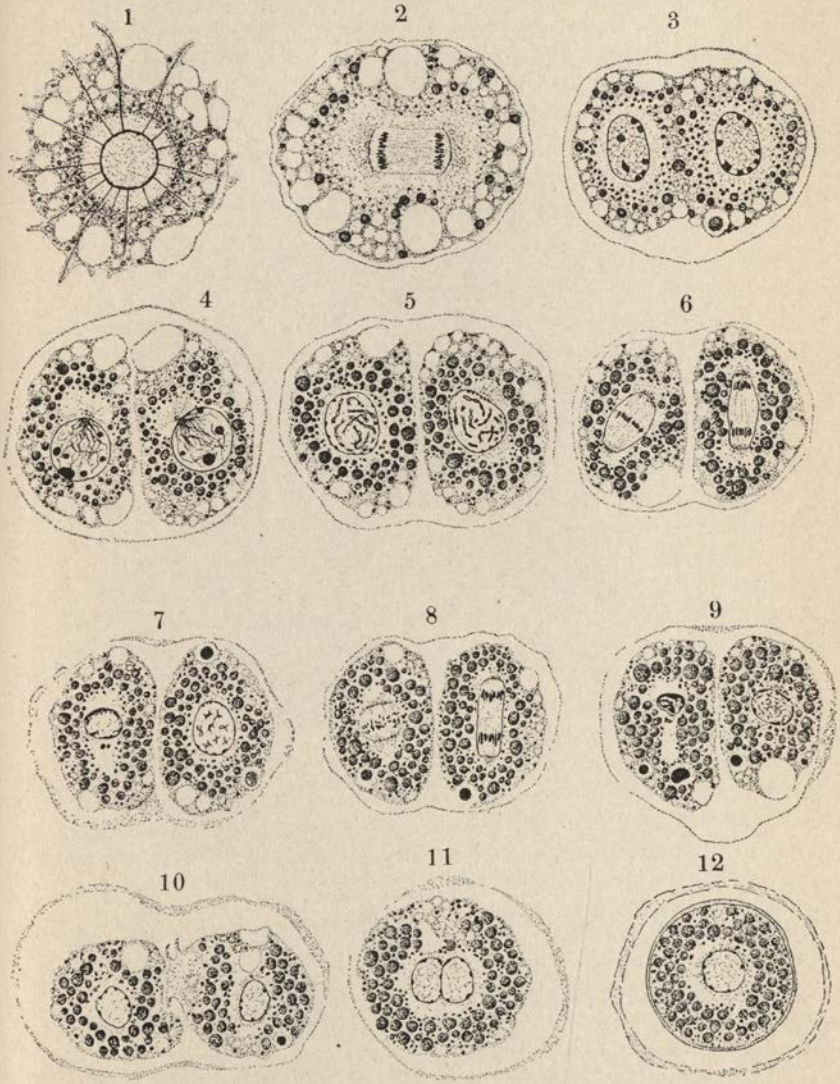

FIG. 209.—*Actinophrys sol*, maturation and copulation of gametes. 1, section of individual prior to fertilization; 2, 3, division of nucleus and cell to form two gametocytes; 4, 5, 6, first meiotic division of the two gametocytes; 7, 8, 9, second meiotic division and formation of gametes; 10, differentiation of the gametes; 11, 12, fusion of cell bodies and nuclei. (After Bělař.)

to Brandt (1885) and Borgert (1900) the same central capsular protoplasm gives rise to anisogametes in the form of two types of flagellated swarmers, but fusion of gametes has not been observed.

A further stage in the manifestation of differentiation at times of maturity is shown by those Protozoa in which the form, character, and size of the fusing gametes are widely different. Here progressive differentiation has followed two general directions resulting, in one direction, in the formation of large, usually quiescent, food-stored cells the macrogametes, in the other direction, in

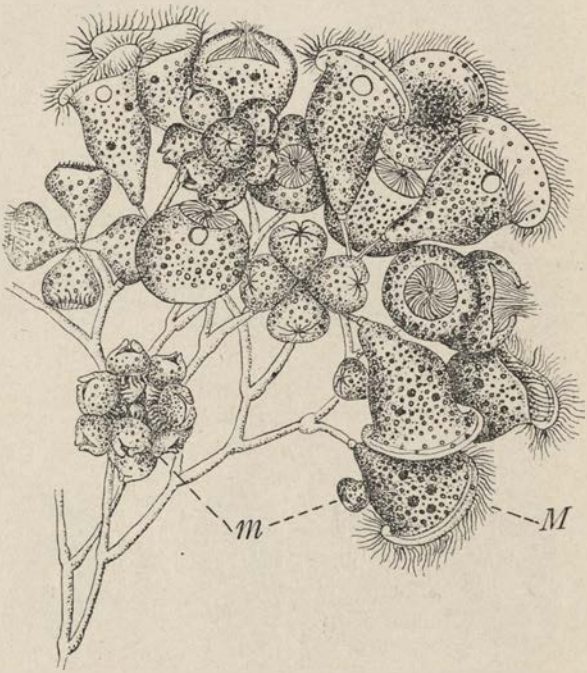

FIG. 210.—*Epistylis umbellaria;* colony with mature macrogametes and microgametes and their fusion (*m*) and (*M*). (After Greeff.)

minute highly motile cells, the microgametes. In these cases furthermore the differences in the gametes may be followed back through the gametocytes for several generations so that cells destined to give rise to macrogametes or to microgametes may be distinguished at an early period.

Examples of this type of anisogamy are practically limited to the Phytomonadida and the Coccidiomorpha. In the Ciliata, however, there is a partial differentiation in this direction in the Vorticellidæ where a larger and attached individual—the macrogamete—is scarcely distinguishable from vegetative agamonts, while the micro-

gametes are one-eighth as large and are formed by three successive divisions of the microgametocytes (Fig. 210). The microgametes always become detached and swim about actively until they perish or meet and fuse with a macrogamete.

Amongst the Phytomonadida, the Volvocidæ illustrate every transition from isogamy to complete anisogamy of egg and sperm, the type being distinguished as oögamy. Here the genera *Eudorina*, *Pleodorina* and *Volvox* show real sexuality, eggs and spermatozoa being formed, in some cases in sexually differentiated colonies. According to the investigations of Goroschankin (1875), Goebel (1882), Chatton (1910) and Merton (1908) male and female colonies are differentiated in *Eudorina* and *Pleodorina*. Egg cells of the female colonies are only slightly different from the usual vegetative cells. In the males the cells become highly modified; all cells of the male colony, with the exception of the most anterior, become gametocytes which divide as though forming daughter colonies but the usual 32 gametes remain in a *Gonium*-like plate. Their color changes from green to yellow and they develop 2 flagella and become much elongated. *Volvox*, in the main, agrees with *Eudorina*. In *V. globator* from 20 to 64 flagella-free, round cells, in *V. aureus* from 1 to 15, are produced in the vegetative half of the mother-colony (Fig. 211). Microgametocytes are variable in number at the generative pole; in *V. globator* the number is small (from 1 to 5), but in *V. aureus*, the cells of an entire hemisphere or even more, may become microgametocytes (Fig. 211, *B*, *D*). In each microgametocyte, plates of microgametes are formed as in *Eudorina*. They are spindle-shape and somewhat spirally bent with a chromatophore at the posterior end and a proboscis-like anterior end with laterally inserted flagella.

Volvox globator is represented by both vegetative and germinal colonies. The former give rise only to parthenogenetic or asexual daughter colonies, the latter are monœcious producing both macro- and microgametocytes. According to some observers the colonies are protandrous making self-fertilization impossible, but others find that self-fertilization is not infrequent (Klein, Overton, *et al.*). In *Volvox aureus* there are pure vegetative, pure female and pure male colonies but the progeny of a colony of any type does not of necessity follow the parental type.

A similar complete differentiation, or oögamy, is shown by the majority of Coccidiomorpha amongst the Sporozoa. In some cases, however, notably in the genus *Adelea*, gamete differentiation is of the same general type as in the Vorticellidæ. In other cases a multitude of minute sperm-like gametes are formed from the microgametocyte while the macrogamete appears like a slightly modified vegetative individual (Fig. 183, p. 427). In *Cyclospora karyolytica*, Schaudinn (1905) maintained that differences shown

by the mature gametocytes could be followed back to the sporozoites from which they came.

In these various cases we find quite variable expressions of differentiation in the protoplasm of a given species. This differentiation appears to be cumulative in the life cycle and the same initial protoplasm through differentiation in two directions may, at matur-

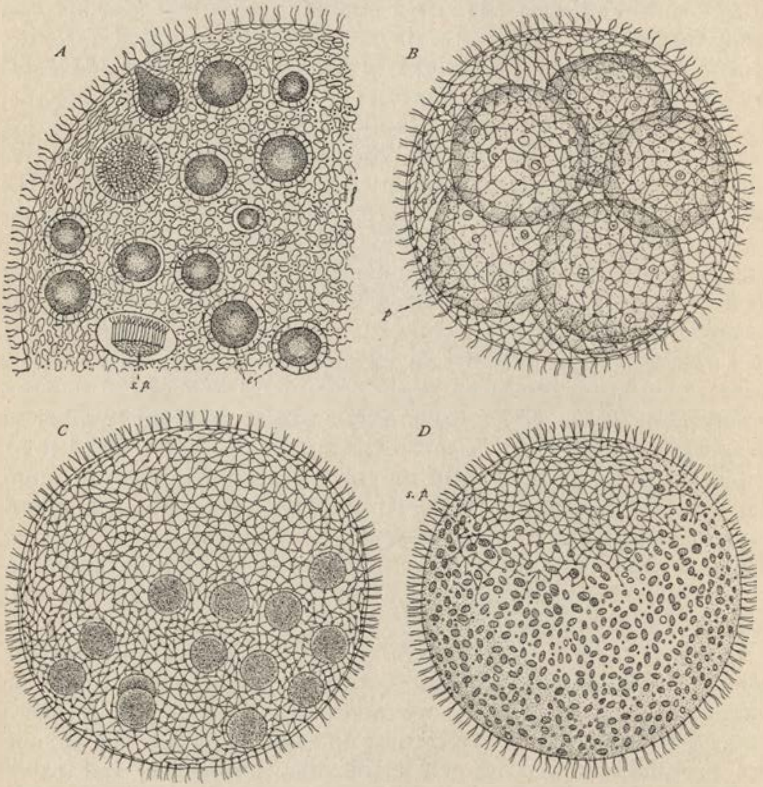

FIG. 211.—*Volvox globator* (*A*) and *V. aureus* (*B*, *C*, *E*) *A*, Sexually mature colony with eggs (*e*, macrogametes), and spermatozoids (sp.). *B*, asexual colony with young agamous daughter colonies; *C*, female colony with macrogametes; *D*, male colony, with many bundles of spermatozoids (microgametes). (From Oltmanns.)

ity, give rise to both types of gametes. If however, the differentiation in two directions is manifested at the very outset of a life cycle in organisms developing from zygotes, one ultimately giving rise only to macrogametes, the other only to microgametes, then we are dealing with a matter of inheritance and not with progressive or cumulative differentiation through metabolic activities. In

such instances, particularly if the differentiations are manifested by structural features whereby one type can be distinguished from the other we are justified in using the term sex in the same sense as used for Metazoa.

Summary.—In the preceding pages an hypothesis has been developed for the purpose of bringing together a large array of disconnected facts in one comprehensive biological generalization. The underlying principle is the irritability of protoplasm as manifested by the phenomena of adaptation. The fundamental organization or particular type and arrangement of the proteins, carbohydrates, salts, and other constituents of living substance, is specific for each kind of organism. Vitality is interpreted as the aggregate of chemical and physical reactions going on between and amongst the diverse parts of the organization and between these and the environment. Adaptation is the response of the organization to unusual conditions. It involves somewhat changed reactions and these in turn, may involve new substances which may or may not be the basis of new morphological elements, but the fundamental organization becomes at least somewhat modified. The inciting causes of such changes may be of environmental or of internal origin. Amongst the latter are new combinations which occur with amphimixis. Here also, are the new substances which are formed as a result of metabolism, particularly of oxidation. These may or may not be labile, *i. e.*, subject to reversal of phase in a physical sense, or to participation in the vortex of vital activities generally. If not labile they become metaplastids and may or may not serve some useful purpose for the organism. If such products of activity are labile new combinations with other substances in the protoplasm are possible and the results are manifested as differentiations.

On this basis we interpret the differentiations which appear with the intake of water and oxygen by an encysted organism (p. 489) or the various activities characteristic of Protozoa during the early phases of the life history. On the basis of changes due to general metabolic activities and due to the specific organization of any particular form, we interpret the drastic alterations which accompany and characterize cell division. These involve the changes in physical condition of the various colloidal substances, such for example, as the increase in permeability due possibly to the accumulation of hydrogen ions, and the absorption of water. They also involve cytolytic activities as indicated by the disintegration and absorption of kinetic elements, of eliminated nuclear chromatin, and division of all the substances active in vitality. The conditions under which these divisional activities are manifested represent inter-divisional differentiations which are reduced through protoplasmic activities at division leaving the organization in a labile

state characteristic of the early inter-divisional period. If the reorganizations effected by these divisional activities are always the same generation after generation, then, on the hypothesis, there is no *a priori* reason why under appropriate environmental conditions, metabolic activities, or vitality, should not continue indefinitely (See Child, Hartmann, Bělař, Jollos, etc.). Such is the explanation that I would give of continued life without fertilization of animal flagellates, aided here possibly, by changes which may take place during the periods of encystment. On the same basis we find an explanation of the long-continued isolation cultures without fertilization of organisms which, under usual conditions, undergo fertilization. Some types of organization are evidently able under appropriate conditions of the environment to return to the same labile organization after each division. Such types would thus have a prolonged asexual cycle, possibly, as Enriques asserts, as long as the observer cares to continue the culture. Here *Eudorina elegans* (Hartmann) and *Actinophrys sol* (Bělař) are conspicuous examples to which should be added the exceptionally long-lived races of Infusoria in the hands of Enriques, and of Woodruff and his pupils. Here also we may add the amicronucleate race of *Didinium nasutum* which Patten (1921) followed through 652 generations in two hundred and thirty-eight days, and one series of *Uroleptus mobilis* which lived through a period of five hundred and ninety-eight days, divided 597 times and had a relative vitality of 110.4 per cent, although such cases may be interpreted as due to peculiar combinations through amphimixis.

If, however, reorganization as effected by division does not leave the protoplasm in its original labile condition, then inter-divisional activity of the progeny starts with a different organization than did the previous generation and this, continued generation after generation produces an accumulative effect. This is manifested by physiological activities and by structural modifications not shown before. The decline in the division-rate for example, may indicate that the living substances are becoming relatively stabile and more and more irreversible in phase as was the case with one race of *Paramecium caudatum* in which the individuals became homogeneous and black in appearance with complete loss of the usual vesicular character (Calkins, 1904, Fig. 212). This particular condition was relieved by the use of electrolytes added to the usual medium (K_2HPO_4, KCl, etc.). In extreme old age in ciliates there is apparently a cessation of the intricate activities involved in cell division. Evidence of this is the tendency to form monsters and the tendency of parts to undergo degeneration, nuclei, motile organs, kinetic elements, etc, in particular.

Between the extremes of youth on the one hand and old age on the other is a condition of cumulative differentiation termed sexual

maturity. In this condition phenomena occur which do not occur earlier and the organization may become visibly altered. Thus gregarines lose their attaching organs and become gamonts; the physical condition of *Paramecium* changes to such an extent that two individuals will fuse on contact at any part of the cortex (I have observed an amorphous group of nine such partially fused individuals); or the phenomena of plastogamy in general are possible under such conditions of differentiation.

With the protoplasm in this latter condition due to continued metabolism further differentiations are possible and, carried out in different directions, lead to specializations characteristic of gametes. As Bütschli first suggested inequalities in division may account for

FIG. 212.—*Paramecium caudatum* in a period of depression and recovery by treatment with salts. (After Calkins.)

the differences in gametes, a possibility indicated by the more irritable anterior region of the ciliates, or by the more active pulsations of the anterior contractile vacuole in *Paramecium*.

When such differentiation progresses to the point of isogamete and anisogamete further metabolic activities and reproduction are stopped, and if fusion is prevented, the gametes die. With the ciliates this is true only of the Vorticellidæ. In other ciliates, differentiations at sexual maturity have not proceeded far enough to seriously affect the general metabolism and power of reproduction. This is demonstrated by experiments with "split" pairs, or separation of two individuals recently united in conjugation, an experiment first performed by Hertwig (1889) and later by Calkins (1904, 1919) and by Jennings (1909). Here an individual thus

separated, continues with the same division-rate that it would have had had it not conjugated. Yet the history of isolation cultures with exceptions noted above, shows that ultimately if conjugation and parthenogenesis are continually prevented, the race, like anisogametes, will die.

This brings us to the consideration of the phenomena, including meiosis and reduction of chromosomes taking place during fertilization, and to the much-discussed matter of the effects of conjugation or fertilization generally on the organization and on vitality. These will be discussed in the following chapters.

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CHAPTER XI.

PHENOMENA ACCOMPANYING FERTILIZATION.

In the preceding chapters we have endeavored to show that continued metabolism leads to changes in the organization of Protozoa whereby phenomena of a cyclical nature in the life history are possible. Among such changes are those which underlie activities at periods of sexual maturity including gamete formation. In the present chapter I wish to consider the activities which take place immediately before, during, and immediately after fertilization, phenomena which are involved in any attempt to interpret the effects of fertilization. Here we have to do with protoplasm which has become so changed in organization that further metabolism is impossible as in highly specialized gametes, or with protoplasm which is so little changed that metabolic activities are still possible. The special problems to be considered in this connection are: (1) The environmental conditions under which fertilization occurs; (2) fertilization types; (3) the internal phenomena of maturation and reduction in number of chromosomes; (4) the immediate metagamic internal activities involved in reorganization; (5) parthenogenesis.

I. THE ENVIRONMENTAL CONDITIONS OF FERTILIZATION.

(*a*) **Ancestry.**—Attempts to analyze the conditions under which fertilization by fusion of gametes, or by conjugation, takes place have been made in relatively few cases. Since the first of such attempts, and the majority of later ones, have to do with the conditions of conjugation in ciliates we may consider these first. Of the three conditions cited by Maupas (1889) as necessary for fruitful conjugation—sexual maturity, diverse ancestry, and hunger—the last one only has to do with environmental conditions. The second condition, however—diverse ancestry—was considered so important by Maupas and has been so frequently called upon in explanation of results obtained by many subsequent investigators, that it cannot be ignored. Maupas found that individuals of the same ancestry either would not conjugate at all among themselves, or if they did the ex-conjugants were weaklings and soon died. He also found that, with other evidences of degeneration, closely related individuals of extreme old age showed a tendency to con-

jugate and that such conjugations always lead to sterile results or to abnormal ex-conjugants which quickly die.

Largely as a result of these conclusions of Maupas an unwarranted importance has been attached to the relationship of gametes, and fertilizations have been described as exogamous, endogamous, autogamous, or pædogamous. Of these the third refers to self-fertilization and the second and fourth to union of closely related individuals. Such terms serve a useful purpose for descriptions but are without significance in the matter of effective fertilization. It is quite possible, however, that a brood of gametes from the same gametocyte will have a common physical and chemical make-up and will not be attracted to one another but will meet and fuse with apparently identical gametes from another gametocyte. This appears to be the case with *Polystomella crispa* according to Schaudinn (1903) and also of gregarines. The significance of ancestry however, appears to be in the matter of mating rather than in that of effective fertilization and belongs to the same group of phenomena as the fact that sperm cells do not unite with sperm cells or eggs with eggs. With Infusoria Maupas' conclusion has not been supported by later observers. Calkins (1904) found that fully as many conjugations between closely related forms of *Paramecium caudatum* were fruitful as between forms of diverse ancestry, and one such ex-conjugant from a closely-related pair, was followed through 379 generations by division. Similar evidence has been furnished by isolation cultures of *Didinium nasutum*, *Paramecium aurelia*, *Paramecium bursaria*, *Stylonychia* sp., *Blepharisma undulans*, *Spathidium spathula*, *Oxytricha fallax*, and *Chilodon cucullus*. With *Uroleptus mobilis* the protoplasm of one individual gave rise to progeny which would conjugate whenever the proper conditions were provided, and the 120 series derived from ex-conjugants from such unions furnish ample proof that the conjugations were fruitful. Such results indicate that Maupas' conclusion regarding the necessity of diverse ancestry was incorrect.

(*b*) **Environment.**—One unmistakable conclusion can be drawn from the many diverse observations and interpretations of the conditions under which fertilization occurs in ciliates, viz., the protoplasmic state with which conjugation is possible is induced in large part, but not wholly, by environmental conditions.

In practice the simplest way to obtain conjugations in ciliates is the method adopted by Maupas. A pure culture of the organism to be tested is allowed to multiply freely in a rich culture medium; a large number of these are then transferred to a smaller container with enough of the original medium in which they had developed to make it unnecessary to add fresh medium. In this second container, conjugations will appear in from twelve to thirty-six hours provided a mixed population is present. In a similar way conjuga-

tion tests are made at regular intervals in all complete isolation culture work. Such tests have been made with *Uroleptus mobilis* every ten to fifteen days.

The usual interpretation of this result is not very enlightening; it runs somewhat as follows: After abundant feeding and active division the large numbers of individuals produced soon exhaust the food, and hunger follows; conditions thus due to hunger result in conjugations provided the organisms are mature. Jennings (1910) qualified this general statement by emphasizing the necessity of a preliminary period of active multiplication in a rich food medium. "The cause of conjugation" he states, "is a decline in the nutritive conditions after a period of exceptional richness that has induced rapid growth and multiplication" (loc. cit. p. 292). All experimenters since Maupas have used this method with more or less success and it appears to be empirically sound. Some observers however, interpret the phenomenon as due exclusively to such purely environmental conditions. Thus Chatton (1921) argues that inanition is indeed an "internal condition" but the lack of food which causes it is an external factor. "Inanition" he says, "is a condition which is practically all that is needed for conjugation; it is an almost certain means of obtaining conjugations in no matter what wild culture, and becomes the chosen technical means of producing them. In current theories, however, conjugation is regarded as independent of the external conditions, inanition playing only an occasional role" (loc. cit. 131). Yet, in a footnote (page 135), Chatton very properly calls attention to the fact that conditions which call forth conjugations in nature do not cease after conjugation is ended. Indeed it is an unwarranted assumption to explain conjugations in nature as induced by a period of rich feeding followed by a period of lack of food, and this in turn replaced by a rich nutrient medium useful to the ex-conjugant. To this extent the method employed in the laboratory to obtain conjugating pairs is entirely artificial. Chatton's reflections and conclusions supporting the view that external conditions are alone responsible for conjugation are included in his excellent description of the structures, division, and conjugation of parasitic ciliates of the family *Nicollellidæ*, particularly *Nicollella* and *Collinella*. In the former the conjugating individuals measure approximately one-fifth of the vegetative forms; in the latter approximately one-half, in both types the conjugating individuals differ in morphological details from the vegetative forms. He interprets these changes as due to the particular part of the digestive tract to which the parasites are carried. Chatton's perplexity and call for further experimental evidence in solving the *raison d'être* of conjugation is justified and the problem will probably remain perplexing so long as external conditions alone, are regarded as the controlling factors.

Of these external conditions other factors than the supply of food may, and apparently do, play a part. Enriques (1903, 1905, 1909, etc.) has long maintained that the phenomena of degeneration and senescence are caused at bottom, not by internal conditions but by external causes, apparently by the accumulation of bacterial products in the medium which poison the organism. Hance (1917) held that they are caused by the concentration of katabolic products derived from the organism and accumulated in the medium. Enriques also makes the statement that upon filtering the liquid in which conjugating forms are present and adding non-conjugating individuals to it, the latter will conjugate; on the other hand a similar liquid with non-conjugating individuals if filtered and used as medium for conjugating individuals, will act as a deterrent to conjugation. Repeated attempts on our part with *Didinium nasutum*, *Paramecium caudatum* and *Uroleptus mobilis* have failed utterly to confirm these results. There is more evidence for his conclusion that salts in the medium are necessary for conjugation, a conclusion based upon his experiments with NaCl, NaBr. and NaI in certain concentrations, on the ciliate *Cryptochilum nigricans*. These particular salts together with strong solutions (1 to 10,000) of $CaCl_2$ and Fe_2Cl_6, produced epidemics of conjugations, while weak solutions of the last two salts inhibited conjugations. Still more extensive experiments along the same line were made by Zweibaum (1912) on *Paramecium caudatum*. Dilute salts $AlCl_3$ in particular added to the medium after a long period of rich feeding, followed by a period of hunger of five to six weeks (sic) produced almost complete epidemics. No salts at all, or very strong salts added to the medium caused no conjugations. These results are certainly suggestive but the experiments should be repeated with carefully controlled material and with some other type than *Paramecium*. With this organism Hopkins (1921) failed to confirm these results. Some rather incomplete and unconvincing experiments by Baitsell (1912) may also be cited in this connection. Two lines of *Stylonychia* from the same ancestral cell, were cultivated on different media; one line on hay infusion, the other on beef extract. Individuals of the former line refused to conjugate while those of the latter line conjugated. From this Baitsell concluded that the determining condition was the medium used. Calkins and Gregory (1914) found that in the same medium some lines would conjugate regularly while other lines from the same ancestral cell would not conjugate at all or conjugate only after nine months of continued culture (see also Hopkins, 1921).

A full consideration of the evidence that has accrued in support of the thesis that external conditions are alone responsible for the onset of conjugation leaves one with the same perplexity that troubles Chatton, Woodruff, and others and calls forth the same

demand for further experimental evidence. Indeed some embarrassing questions based upon what we already know must be answered: If it is environment alone what are the external conditions responsible for the formation of the gametes in Coccidiomorpha, Gregarinida, Foraminifera and Phytomonadida? Or in the ciliates what is the explanation of the failure of external conditions to induce conjugations in some lines and not in others? Or why will the same external conditions fail with youthful forms when they are successful with older (mature) forms?

In practically any epithelium deeply infected with coccidia adjacent cells contain vegetative stages of the organism, agamont stages in reproduction, gametocyte stages of both kinds, and nearby are zygote stages. If conditions of the infected host cell are responsible for the different phases it must be a very delicate difference that calls out asexual reproduction in one and gamete formation in another, and all within the radius of a single field of the microscope. If products of degeneration of an infected host cell cause gametocyte differentiation in one organism why do not the products of the cell next to it produce a similar effect on its contained organism instead of which we find the latter reproducing asexually? The conception of external factors as the sole cause of protoplasmic changes leading to fertilization must be very elastic to cover such cases. Why are not all malaria parasites transformed into gametocytes if the blood is the determining factor? *Plasmodium vivax* taken into the gut of the mosquito should be transformed into gametocytes producing gametes instead of which only gametocytes already formed produce gametes while agamonts are apparently digested; and in the blood of man or birds these gametocytes circulate with the vegetative forms and with agamonts. Surely in these parasitic forms, granted that external conditions may be provocative some internal condition of the organism nevertheless predetermines the action of the environmental stimuli.

With ciliates every experimentalist knows that in pure line work conjugation tests are sometimes successful, sometimes not. Jennings (1913) noted this in different races of *Paramecium;* Woodruff for several years was unable to obtain a single pair from his famous culture of *Paramecium aurelia,* although ultimately they did conjugate; Calkins and Gregory (1914) cultivating the first eight individuals from an ex-conjugant of *P. caudatum* in pure lines, found that conjugations were abundant in certain lines whenever a test was made, while other lines remained negative at every test until the race was many months old. Similar tests made with any series of *Uroleptus mobilis,* and by test we mean a period of rich feeding followed by hunger, is negative if the organisms are young, positive if the organisms are mature (Fig. 204, p. 491). All of these facts, and the literature contains many other similar cases,

indicate that environmental stimuli are without effect in producing conjugations unless the protoplasm is in a condition where such conjugations are possible. Indeed, when fully mature, *i. e.*, when the protoplasmic conditions are just right for conjugation, union will take place in a rich food medium and without the transition from full nourishment to hunger. This phenomenon is abundantly illustrated in the records of *Uroleptus mobilis* and in my records of *Paramecium caudatum*, *Blepharisma undulans*, or of *Didinium nasutum*. There is little information as to the exact nature of these protoplasmic conditions prior to conjugation. Zweibaum (1922) gives good evidence to show that the quantity of glycogen in the cell is reduced to a minimum at this period, the large drops of neutral fat disappear while small droplets of another type make their appearance together with some cholesterine ester and large quantities of what was interpreted as fatty acids. These are probably effects of inadequate food material, for the observer obtained similar results with Paramecia under conditions of starvation which were not followed by conjugation.

II. INTERNAL CONDITIONS AT THE PERIOD OF FERTILIZATION.

In the last analysis both internal and external conditions play their respective parts in protoplasmic preparations for conjugation. Without external stimuli, without oxygen and food, vitality would soon cease; with them, vitality manifested by metabolism and reproduction will continue. With metabolism, however, the protoplasmic make-up is constantly changing and these changes are shown by the general reactions and by the organization (see Chapter IV). According to Hertwig, 1908, Popoff (1908), and Rautmann (1909), the changes thus brought about lead to disturbances of the normal ratio of nucleus to cytoplasm (Kernplasmaverhältnis) and lead to conjugations whereby the normal relation of nucleus to cytoplasm is regained. Whatever the changes due to metabolism are in a given case the conclusion is forced upon us by the mass of evidence that given external conditions will provoke conjugations at one period of the life cycle and will have no effect in producing them at another period, while at the critical period of maturity external conditions may be entirely negligible as they appear to be in the Coccidiomorpha and in gamete-forming organisms generally. Here protoplasmic and not external conditions control the issue. There is some significance in the fact that encystment (with endomixis) is induced by the same external conditions as is conjugation. Mengheni (1913) found that *Stylonychia* will not encyst if food is abundant but that hunger and low temperature are necessary conditions. With *Uroleptus mobilis* conjugation and encystment tests

are made in exactly the same way and in some tests conjugating pairs and encysted forms are present simultaneously.

In the case of *Uroleptus mobilis* a mass culture of young individuals shows no tendency to agglomerate, the cells are distributed more or less uniformly in the culture. In similar mass cultures of individuals approaching maturity agglomeration in dense groups is highly characteristic. Such cultures may show no conjugations, but a mass culture made with the progeny of the same individuals a week later will show not only the initial agglomerations but epidemics of conjugation as well (Calkins, 1919).

This phenomenon of agglomerations indicates something of the nature of an attraction that increases in intensity as the organisms approach maturity and have a bearing on the problem of mating. What is it that brings two gametes together or two apparently similar ciliates? There is some evidence that the attraction is of a chemiotactic nature as illustrated by the often quoted experiments of Pfeiffer with malic acid and fern spermatozoids. Two citations from Engelmann (1876) may illustrate this phenomenon with ciliates of the genus *Vorticella:* "The buds, at the beginning, swarmed about with constant and considerable rapidity rotating the while on their axes but moving more or less in a straight line through the drop. This lasted from five to ten minutes or even longer without any special occurrence. Then the scene suddenly changed. Happening in the vicinity of an attached *Vorticella* a bud quickly changed its direction with a jerk and approached the larger form, fluttering about it like a butterfly over a flower and gliding over its surface here and there as though tasting. After this play, repeated upon several individuals, had gone on for several minutes, the bud finally became firmly attached." Again: "I observed another performance still more remarkable. A free-swimming bud crossed the path of a large *Vorticella* which had become free from its stalk in the usual manner and was roaming about with great activity. At the instant of the meeting, there was no trace of a pause, the bud suddenly changed its direction and followed the *Vorticella* with great rapidity. It developed into a regular chase which lasted about five seconds during which time the bud remained about $\frac{1}{15}$ of a millimeter behind the *Vorticella* although it did not become attached for it was lost by a sudden side movement of the larger form" (loc. cit., p. 583). Another illustration taken from the observations of Schaudinn (1900) on the mating of gametes of *Eimeria schubergi,* suggests an action analogous to that of attraxin as described by F. R. Lillie in sea-urchin eggs. During the maturation of the macrogamete of *Eimeria schubergi,* the "karyosome" is cast out of the nucleus breaks into fragments and the fragments are extruded from the cell, remaining however, attached to the periphery. The microgametes swim aimlessly about and are not

attracted to the macrogamete until after these fragments are eliminated, but as soon as the granules appear on the surface the microgametes move toward them in the most direct path (loc. cit., p. 257). Zweibaum (1922) observed that the glycogen content is fundamentally different in the two individuals of a conjugating pair of *Paramecium*, which may be significant in this connection.

While chemiotaxis may underlie the phenomena described above, an equally intelligible interpretation might be drawn on the basis of differences in potential of a magnetic nature. Two individuals of *Uroleptus mobilis* about to conjugate, circle about one another, twist and turn but do not become separated; finally they become lightly fused by the extreme anterior parts of their peristomes and the zone of fusion ultimately extends about half way down the peristomes. In the early stages, as with *Paramecium*, the two individuals can be separated without injury to either ("split pairs") but later the two protoplasms are welded into one, forming a protoplasmic bridge between the individuals. Experiments in cutting apart the two fused individuals have shown that immediately after contact and initial fusion the complete series of maturation divisions proceeds as though the separated individuals were still in conjugation (Calkins, 1921) and similar cutting at any time during the period of conjugation, does not alter the course of the internal and consecutive processes. Ultimately reorganization of the individual follows in due course and the subsequent happenings are exactly like those of an ex-conjugant. These experiments indicate that the phenomena of maturation and of reorganization which characterize fertilization in *Uroleptus mobilis* are of the nature of an "all or none" series of reactions and when once started they go through to the end without deviation. It also appears that the stimulus which sets in motion this chain of processes is received at the time of initial contact and is mutually received by both conjugating individuals. It thus appears to be less of a chemical reaction than a physical one and has many of the attributes of a surface contact phenomenon between surfaces of different electrical potential.

III. THE PROCESS OF FERTILIZATION.

The actual process of fusion, with the exception of fertilization by conjugation, furnishes little material for descriptive purposes, two cells come together and fuse, probably with cytolysis of the contiguous cell membranes. In hologamic forms of ciliates (*e. g.*, in *Balantidium coli* according to Brumpt) which are extremely rare, two individuals come together as in pseudo-conjugation of gregarines; they secrete a common cyst membrane and then fuse completely.

In isogamic and often in anisogamic fertilization, fusion begins

as a rule with union of the flagellated ends, if the gametes are motile as in *Scytomonas*, *Polytoma*, *Polystomella* and gregarines, etc. (Fig. 96, p. 211). In *Actinophrys sol* (Fig. 209) according to Bělař, one of the fusing individuals develops a pseudopodium which unites first with the other cell.

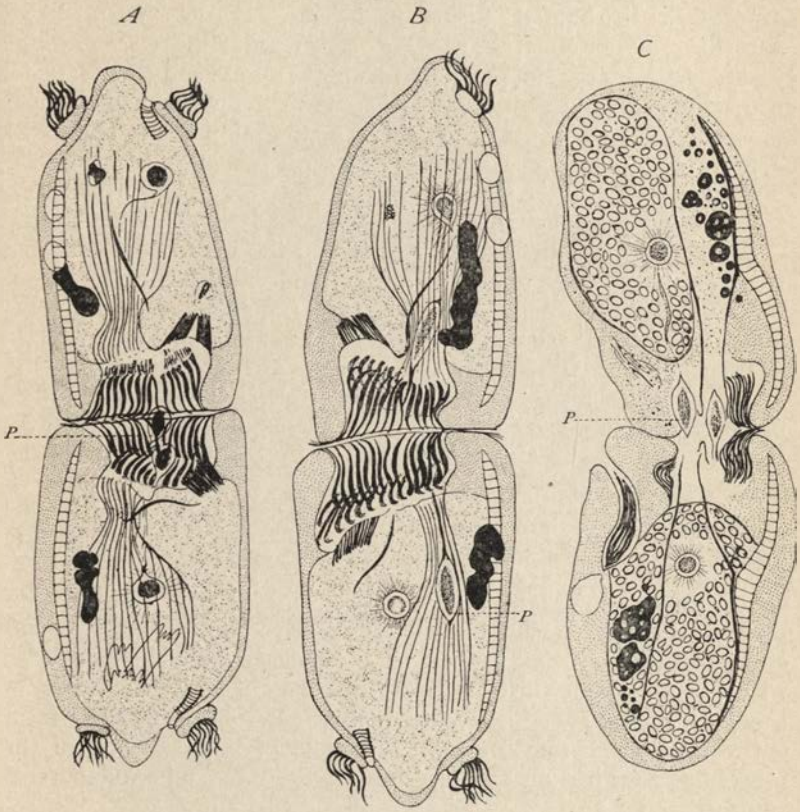

FIG. 213.—*Cycloposthium bipalmatum* and *Diplodinium triloricatum;* conjugation. *A*, *Cycloposthium* with the two migrating pronuclei in the chamber formed by the two peristomial spaces; *B*, same, the two migrating pronuclei have passed from the peristomial chamber into the gullets; *C*, *Diplodinium*, with migrating pronuclei in the peristomial chamber in their passage from one individual to the other; *p*, pronuclei. (After Dogiel.)

With anisogamic fertilization the microgamete is usually motile, the macrogamete is stationary and is sought by the microgamete and the same is true also of oögamic fertilization. In some cases the macrogamete is smaller than the migrating microgamete (Fig. 183, p. 427). In the *Vorticellidæ* the macrogamete remains attached while the microgamete is free-swimming.

In hologamous fertilization by conjugation there is no universal mode of fusion. In the majority of ciliates with adoral zones the fusion area is usually the anterior region of the peristomial furrow, the mouth as a rule being involved (*e. g.*, Fig. 213). In exceptional cases the mouth itself is involved in the protoplasmic bridge between the two conjugants (*Paramecium* sp. *Didinium nasutum*, *Spathidium spathula*). In *Stentor* fusion is lateral. Dogiel (1923, 1925), describes an interesting case of conjugation in *Cycloposthium bipalmatum*. Here the two individuals are united end to end, fusion occurring at the borders of the peristomes, leaving the membranelles of the adoral zone intact in a common conjugation cavity (Fig. 213). The wandering pronuclei are provided with tails and, spermatozoa-like, break through the anterior wall and into the conjugation cavity from which each enters the other conjugant by way of the mouth.

A Meiotic Phenomena.—In relation to the union of cells in fertilization the meiotic phenomena may be divided into three types: (*a*) Conjugant meiosis, or maturation processes occurring only after union of the participating cells; (*b*) gametic meiosis (Wilson) or types in which the maturation processes are antecedent to union; and (*c*) zygotic meiosis (Wilson) characteristic of forms in which meiotic divisions occur in the zygote subsequent to the fusion of the nuclei. The first of these is illustrated by conjugating Infusoria; the second by the great majority of types in which fertilization is accomplished by permanent fusion of gametes; and the third by a few known cases among the Sporozoa and in the flagellate *Chlamydomonas*.

(*a*) **Conjugant Meiosis.**—In mature ciliates the protoplasmic organization is such that the stimulus received on contact is apparently all that is needed to start up the nuclear activities associated with the phenomena of chromosome reduction and preparation of the pronuclei. These activities furthermore, have to do almost entirely with the micronuclei. Macronuclei take no part in the process of fertilization but are important in the subsequent reorganization.

With one or two exceptions (*Trachelocerca phœnicopterus*, *Spirostomum ambiguum*, etc.) all of the free-living ciliates thus far described agree in the general course of their maturation phenomena. Maupas (1889) the first to make a comparative study of different ciliates during conjugation, described eight successive phases of the process which are still applicable to practically all ciliates. Of these, Phase A, is characterized by the swelling and early changes of the micronucleus; Phase B, is the period of the first meiotic or maturation division; Phase C, the period of the second meiotic division; Phase D, the third nuclear division resulting in the formation of the pronuclei; Phase E, the period of interchange and union of pronuclei; Phase F, the period of the

first metagamic nuclear division; Phase G, of the second metagamic division and Phase H, the period between the second metagamic nuclear division and the first division of the reorganized cell.

The first four of these phases have to do with the phenomena of maturation, the last four with the process of reorganization of the individual. In *Trachelocerca phœnicopterus* this succession of stages according to Lebedew (1908) is entirely absent and fertilization follows quite a different course (page 375). Also in *Euplotes charon* and *Euplotes patella* according to Maupas there is a slight variation in the usual sequence in that an anomalous, additional or preliminary division of the micronucleus takes place in each conjugant prior to the first of the two maturation divisions. In the Peritrichida also a similar preliminary division occurs but in these cases it is limited to the microgamete, the macrogamete following the usual history (*Vorticella monilata, V. nebulifera* Maupas; *Carchesium polypinum* Maupas, and Popoff, 1908; *Orphrydium versatile* Kaltenbach, 1915; and *Opercularia coarctata* Enriques 1907). In the Ophryoscolecidæ according to Dogiel (1925) similar progamous nuclear divisions are followed by division of the cells resulting in much smaller conjugating individuals.

If more than one micronucleus is normally present in the ciliate the first meiotic division usually takes place in all of them and the second division may occur in all, or one or more of the products of the first division may be absorbed in the cell. Some multiple micronuclei have been described in conjugating forms of *Paramecium aurelia* (Hertwig, 1889), *Onychodromus grandis* (Maupas, 1889) *Stylonychia pustulata* (Maupas, 1889; Prowazek, 1899) and *Oxytricha fallax* (Gregory, 1923) each individual having 2 micronuclei. Two or 3 micronuclei are present in conjugating *Didinium nasutum* (Prandtl, 1906); 2 to 4 in *Uroleptus mobilis* (Calkins, 1919); 4 or 5 in *Blepharisma undulans* (Calkins, 1912) and 16 to 18 in *Bursaria truncatella* (Prowazek, 1899).

1. *Phase A. The Prophase Stages of the First Meiotic Division.*— In many ciliates in which the history of maturation has been followed there is very little to distinguish the first meiotic mitosis from the usual vegetative divisions beyond a slight swelling of the micronucleus, fragmentation of its homogeneous chromatin and formation of its chromosomes. This appears to be the case in *Loxophyllum meleagris* (Maupas, 1889), *Spirostomum teres* (Maupas, 1889), *Euplotes patella* (Maupas, 1889), *Colpidium colpoda* (Hoyer, 1899), and in *Blepharisma undulans* (Calkins, 1912). In the case of *Colpidium colpoda* Hoyer (1899) described a typical tissue-cell spireme but this is so exceptional among ciliates that it cannot be accepted without confirmation.

In the majority of ciliates this first meiotic mitosis is markedly

different from somatic mitoses. In different species of *Paramecium* (*caudatum*, *aurelia* and *bursaria*) a typical prophase stage occurs in the form of a crescent derived from the homogeneous micronucleus which first draws out in the form of a long cylinder (Fig. 214). In *Chilodon uncinatus* the micronucleus draws out into a long comma-shaped band and in *Cryptochilum nigricans* (Maupas, 1889) *Vorticella monilata* and *Vorticella nebulifera* (Maupas) and in *Opercularia coarctata* (Enriques, 1907) a similar chromatin rod extends in some cases the entire length of the cell.

Still another type of prophase, the "candlelabra" (Collin, 1909) or "parachute" nucleus (Calkins, 1919) is found in *Onychodromus grandis* (Maupas), *Bursaria truncatella* (Prowazek, 1899), *Didinium*

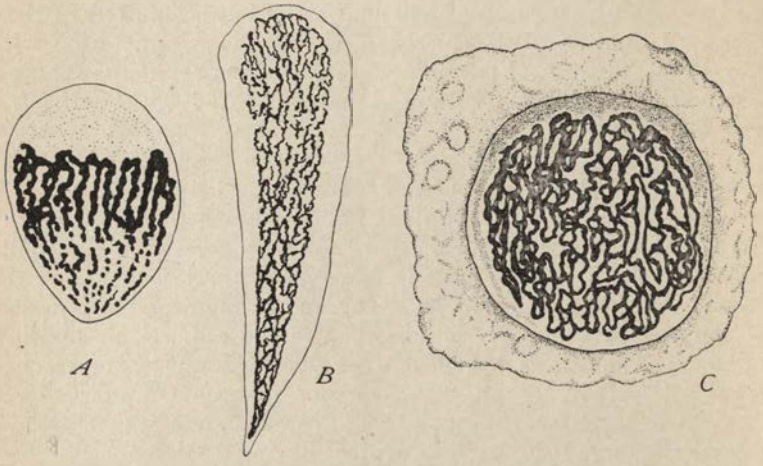

FIG. 214.—Micronucleus of *Paramecium caudatum* in the prophases of the first meiotic division. *A*, Early stage in the formation of chromosomes; *B*, elongation of the nucleus prior to crescent formation; *C*, metaphase of the first division. Dehorne describes the entire chromatin aggregate as forming one highly convoluted chromosome. (After Dehorne.)

nasutum (Prandtl, 1906), *Anoplophrya branchiarum* (Collin, 1909) *Oxytricha fallax* (Gregory, 1923) and *Uroleptus mobilis*. In these cases the nucleus swells to two or three times the usual diameter with the compact chromatin at one pole (Figs. 36, 227). In *Uroleptus mobilis* there is an endobasal body within the nucleus; this divides, one-half passing to the periphery of the nucleus at the pole opposite the chromatin mass while the other half remains with the chromatin (Fig. 36). The distal centrosome is the focal point of the spindle fibers which spread out from it to the fragmenting chromatin mass and forms one pole of the mitotic spindle.

In the transformation of the crescent type of prophase Maupas, Hertwig and Hamburger all agree that the spindle is formed by the

shortening of the long axis of the crescent. Calkins and Cull (1907) and Dehorne (1920), however, find that the division center or achromatinic substance which forms the poles of the spindle

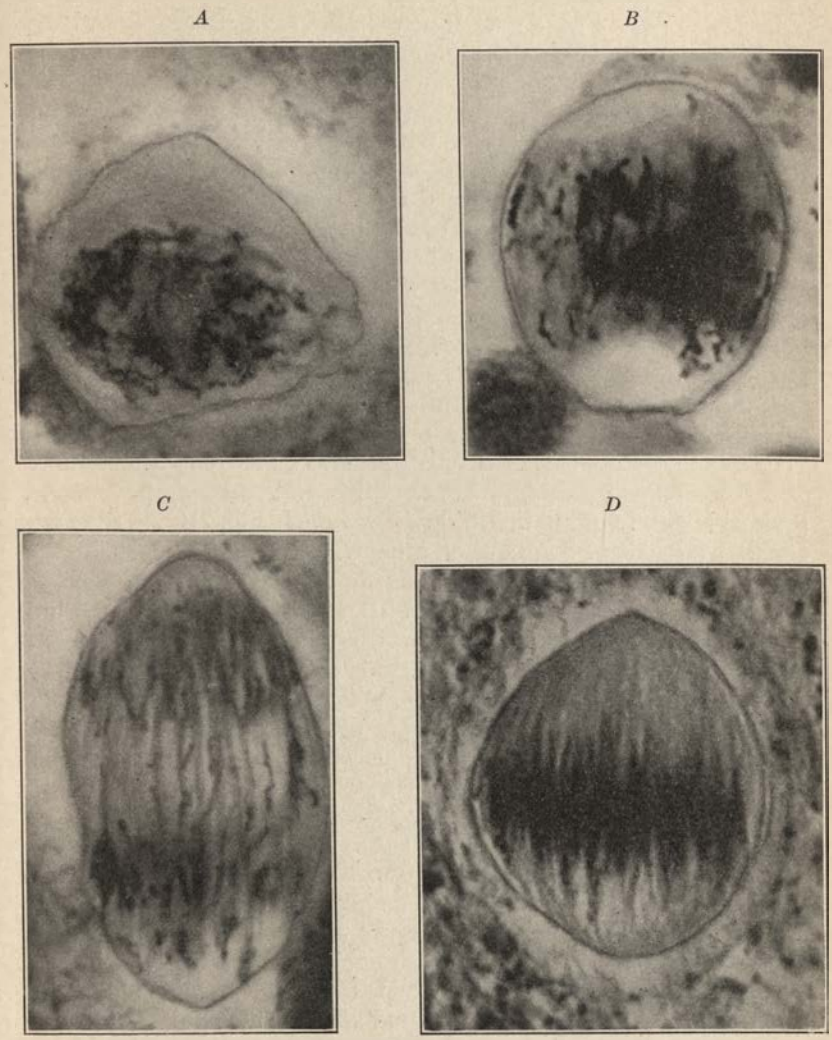

FIG. 215.—*Paramecium caudatum; A, B, C,* stages in the first meiotic division during conjugation; *D,* prophase of second meiotic division. (After Calkins and Cull.)

migrates from its apical position in the crescent to the center of the convex side, and that this new position marks one pole of the spindle (Fig. 215).

In the parachute type the second pole is formed by the outgrowth from the chromatin mass, of a second pole similar to the first, the chromatin granules thus being left in the nuclear plate position or center of the spindle figure (Fig. 36, p. 78).

2. *Phase B. The First Meiotic Division.*—Exact knowledge of the formation of chromosomes and their division is scanty, due in part to the large number of chromosomes and to their small size. Maupas (1889) made no attempt to enumerate the chromosomes; nor did he describe their formation beyond the brief account of the fragmentation of the homogeneous chromatin masses of the micronuclei. Hertwig (1889) believed that there were 8 or 9 chromosomes in *Paramecium aurelia* basing his view not on the chromosomes but on the number of fibers which he could distinguish in the connecting strand between the two daughter nuclei. Later observers have found that the number in all species of *Paramecium* is much greater than this running up to more than one hundred. Dehorne (1920) on the other hand, finds no chromosomes at all, the chromatin being in the form of a continuous single looped thread which divides by transverse division (Fig. 214. Cf. Fig, 215).

In more favorable types of ciliates than *Paramecium* the number of chromosomes has been made out with some degree of accuracy. Prandtl (1906) found 16 in *Didinium nasutum* (Fig. 216). Prowazek (1899) was a little in doubt whether there were 12 or 13 in the nuclei of *Bursaria truncatella*, but described 6 chromosomes in *Stylonychia pustulata*. Stevens (1910) described 4 chromosomes in *Boveria subcyclindrica* but gave no details of their formation or reduction. Enriques (1908), confirmed by MacDougall (1925) found 4 in *Chilodon uncinatus;* Popoff (1908) 16 in *Carchesium polypinum*; Enriques (1907), the same number in *Opercularia coarctata*, and Collin (1909), 6 chromosomes in *Anoplophrya branchiarum*.

Hamburger (1904) is a bit hazy in her account of the origin of the chromosomes in *Paramecium bursaria*. The late stage in the crescent is regarded by her as a spireme from which the chromosomes are formed as short curved or V-shaped rods. Calkins and Cull (1907) found that the chromosomes of *Paramecium caudatum* are derived from a synezesis stage which precedes the crescent and that the chromosomes are already divided at the stage which had generally been regarded as the metaphase. According to this account the metaphase stage occurs during the metamorphosis of the crescent into the spindle so that the latter when formed is in the early anaphase stage (Fig. 215). Dehorne (1920) thinks these chromosomes are due to cutting of the coiled thread by the knife.

In other ciliates the chromosomes are formed by the union of chromomeres which are derived by fragmentation of the homogene-

ous chromatin of the resting micronucleus. The process is completed at the parachute stage and the definitive number is present by the time the second pole of the spindle is completed. In *Uroleptus mobilis* when diffusion of the granules has apparently reached

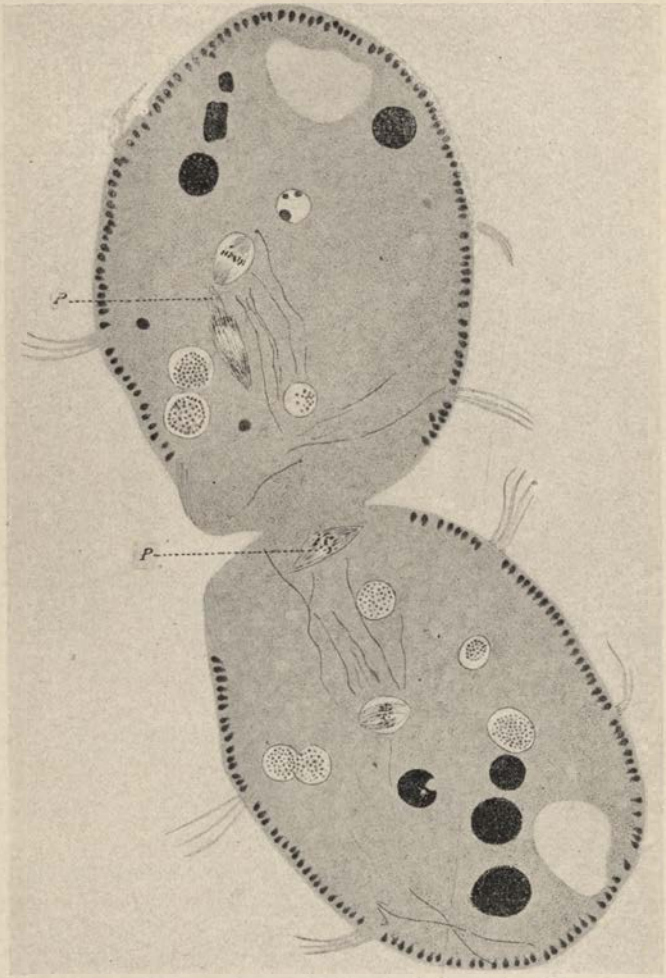

FIG. 216.—*Didinium nasutum*, section of conjugating individuals. Second meiotic division of the nuclei (*P*). (Original.)

its limit, there are from 24 to 28 chromomeres (Fig. 36). Prandtl's figures show that there are approximately 32 in *Didinium nasutum*. Enriques (1908) and Collin (1909) have described a similar fragmentation of the comma-shaped chromatin rod of *Chilodon uncinatus*

and of the homogeneous chromatin mass of *Anoplophrya branchiarum*, the granules of chromatin collecting in the center of the first maturation spindle. In *Didinium*, *Chilodon* and *Anoplophrya* these granules fuse until a definite number of chromosomes result—

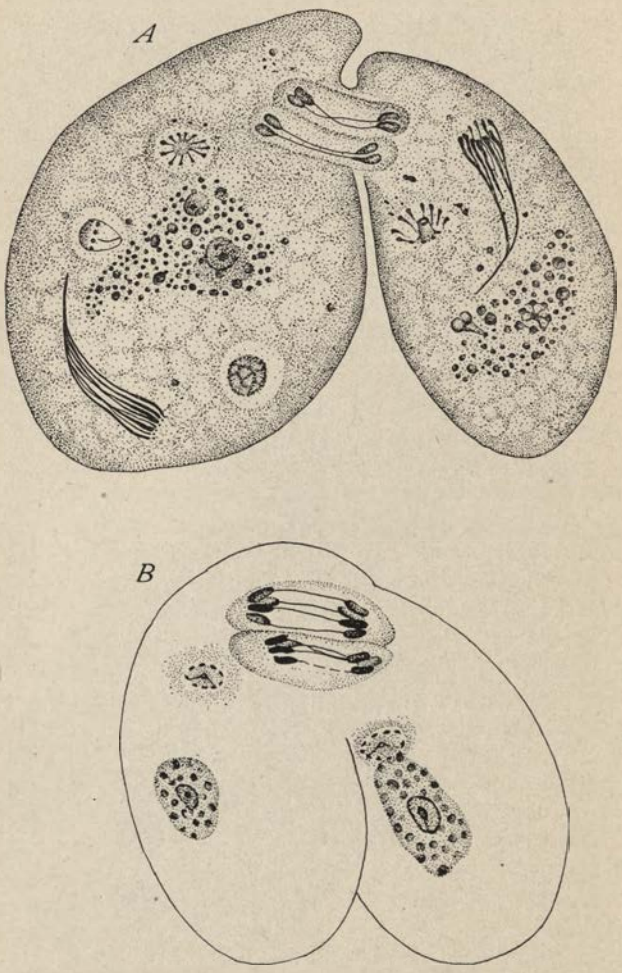

FIG. 217.—*Chilodon uncinatus*. Third division and interchange of nuclei of diploid (*A*) and tetraploid (*B*) stock. (After MacDougall.)

16 in *Didinium*, 4 in *Chilodon* (8 in the tetraploid form found by MacDougall, 1925), and 6 in *Anoplophrya* In *Uroleptus* a similar fusion of granules results in 8 chromosomes (Fig. 36, p. 78). Kofoid and Swezy (1919) described the lateral fusion and reduction in number of chromosomes from 52 to 26 in the vegetative division

(?) of *Trichonympha campanula*. This is incomprehensible on any interpretation of these nuclear elements as chromosomes in reduction but may be explained as pseudo-reduction and without any connection with meiosis.

In few ciliates in which the number of chromosomes can be counted, does this first division result in reduction to one-half the number. Gregory (1923) gives evidence that this is the case in *Oxytricha fallax* and MacDougall in *Chilodon uncinatus* (Fig. 217). In *Paramecium* the chromosomes are short rods too numerous to count. According to the earlier view in regard to the origin of the first spindle from the crescent it was generally assumed that the first division is transverse. The rods are double, however, when formed and the first division is evidently the separation of these two parts, but whether or not it is a reduction division cannot be determined. According to Dehorne (1920) there is no reduction at any stage as there are no chromosomes.

FIG. 218.—*Uroleptus mobilis*. The second meiotic division and reduction in number of chromosomes during conjugation. (After Calkins.)

3. *Phase C. The Second Meiotic Division.*—Prior to Prandtl's work on *Didinium* there were no conclusive observations on the reduction of chromosomes in ciliates. He found that the 16 chromosomes characteristic of the first maturation division become reduced to 8 with the second division. Since his work appeared there have been a number of authentic observations along the same line. Thus Enriques (1907) found a reduction in number from 16 to 8 chromosomes in *Opercularia coarctata* and the same observer (1908) described a reduction from 4 to 2 in *Chilodon uncinatus* (Fig. 217), reduction occurring at the second division. Other cases of the same type are *Carchesium polypinum* (Popoff, 1908) with reduction from 16 to 8; *Anoplophrya branchiarum* (Collin), from 6 to 3; and *Uroleptus* (Calkins 1919) from 8 to 4 (Fig. 218). In all

cases the second meiotic division appears to be unaccompanied by any of the preliminary activities which characterize the first division. In some the nuclei do not return to a resting condition between the two divisions but in other cases, *e. g.*, *Chilodon* (MacDougall, 1925) the second spindle forms from a resting nucleus.

In ciliates with a multiple number of micronuclei the number participating in the second division appears to bear no constant relation to the number derived from the first division. In cases having but one micronucleus in the vegetative stages the numerical relations are fairly constant, two spindles in the second meiotic division being the rule. There are, however, some exceptions. Thus in *Paramecium bursaria* according to Hamburger (1904) one of the nuclei formed by the first division degenerates without forming a spindle so that only one nucleus undergoes the second division. Other exceptions are found in *Euplotes patella* in all *Vorticellidæ* and *Ophryoscolecidæ* examined up to the present time. Here the micronucleus undergoes one or more preliminary mitoses prior to the first meiotic division. In *Vorticellidæ* this unusual division occurs only in the microgamete while the macrogamete follows the usual history of uninucleate forms.

In ciliates with two micronuclei both undergo the first maturation division. According to Prowazek (1899) the 4 resulting nuclei of *Stylonychia pustulata* divide again thus forming 8 products at the second division. According to Maupas (1889), however, 2 of the first 4 nuclei of *Stylonychia pustulata*, and of *Onychodromus grandis* as well, degenerate so that only 2 second maturation nuclei are formed. Gregory's (1923) observations indicate that a variable number take part in the second division of *Oxytricha fallax*.

In forms with many micronuclei in the vegetative stage there seems to be no general rule as to the number which undergo a second division. Prandtl found a variable number in *Didinium nasutum*; Prowazek a large number in *Bursaria truncatella*, and Calkins a variable number in *Uroleptus mobilis*, while 1 and 4 nuclei are rarely found, 2 or 3 are characteristic.

In summing up the accumulating evidence on meiotic phenomena in the ciliates the conclusion may be drawn that the history in the main is similar to the history of meiosis in Metazoa. Chromosomes of definite number are characteristic of each species and this number is reduced to one-half during one or the other of the two divisions. There is the same difficulty with these Protozoa that is encountered with Metazoa in regard as to which of the two divisions is the reduction division in the sense of separation of whole chromosomes. The important fact is that the number of chromosomes is halved; it is less important, indeed relatively unimportant, to know which of the two divisions actually brings it about.

4. *Phase D. The Third Division. Pronuclei Formation.*—A third division of the nuclei subsequent to reduction in number of chromosomes is characteristic of all ciliates in which fertilization has been carefully studied. It is extremely difficult to interpret this final division which gives rise to the pronuclei (see *infra* p. 542). In the majority of cases it appears to be a transverse division which, if judged by Metazoa, would make it a second reduction division. One of the products is a wandering pronucleus which migrates, the other is a stationary pronucleus which ultimately fuses with the migratory pronucleus from the other individual. There is some evidence that the migrating pronucleus is equivalent to a spermatozoön (Dogiel, 1925).

The third division spindles are always characteristic and different from the spindles of the meiotic divisions. Not only are they frequently heteropolar, but the late telophase state is characterized by long connecting strands of nuclear substance (Fig. 31, p. 71). There is no uniformity in regard to the number of nuclei to undergo this third division although only one of the dividing nuclei provides the two functional pronuclei. *Anoplophrya branchiarum, Paramecium caudatum, Chilodon uncinatus, Colpidium colpoda, Leucophrys patula, Glaucoma scintillans, Loxophyllum meleagris, Spirostomum teres, Bursaria truncatella, Blepharisma undulans, Boveria subcylindrica, Lionotus fasciola,* and in the *Vorticellidæ,* only 1 nucleus undergoes this third division. In *Onychodromus grandis Stylonychia pustulata,* and *Euplotes patella,* 2 nuclei, in *Oxytricha fallax* (Gregory) 2 or 3, and in *Uroleptus mobilis,* 2, 3 or 4 nuclei, undergo the third division.

Prandtl (1906) was the first to note a difference in size between the wandering and the stationary pronuclei (*Didinium nasutum*), Calkins and Cull (1907) described a similar difference in pronuclei of *Paramecium caudatum* and were able to trace this difference back to a heteropolar third division spindle. In other cases there seems to be no characteristic difference in size between the two pronuclei although other differences may be evident. Thus Maupas noted the presence of a dense aggregate of cytoplasmic granules at the forward pole of the advancing pronucleus of *Euplotes patella* and Prandtl, more pronounced astral radiations about the wandering pronucleus of *Didinium nasutum.* In *Uroleptus mobilis* such radiations are absent but a fairly homogeneous condensed "sphere" of cytoplasmic substance precedes the wandering pronucleus in its migration. (Fig. 219).

What is the significance of this third division? The answer can be only speculative at the present time. The absence of definite chromosomes in some cases, *e. g.*, *Paramecium,* and the occurrence of heteropolar mitotic figures lend some support to the view that it is a differential division whereby male chromatin, as suggested

by Schaudinn (1904) is separated from "female" chromatin, the balance between the two being established by union of the wandering and the stationary pronuclei. Such an hypothetical balance would be maintained if there were no interchange of pronuclei and the third division does not take place, a condition realized in what Woodruff and Erdmann (1914) called endomixis (see p. 540). Experimental evidence leading to definite conclusions has not yet been advanced. Calkins (1921) made an attempt in this direction by cutting conjugating pairs of *Uroleptus mobilis* in such a way that

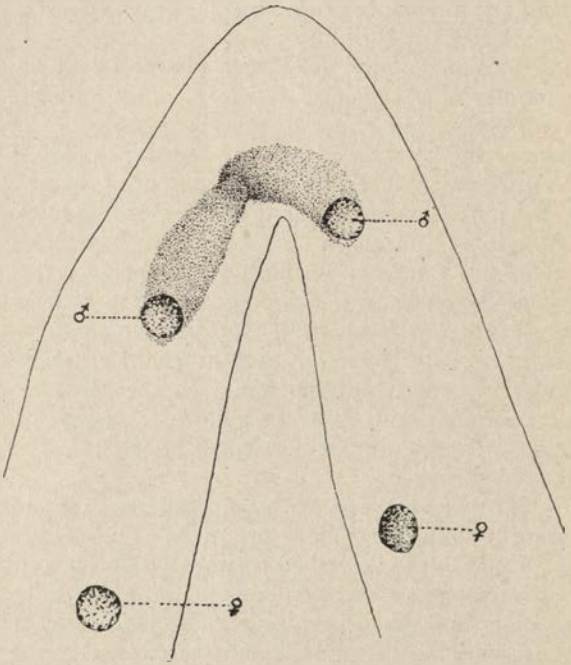

FIG. 219.—*Uroleptus mobilis*, conjugation. The interchange of pronuclei, each preceded by a characteristic "attraction sphere." (After Calkins.)

the two migrating pronuclei were removed while the two individuals, now separated, possessed only the stationary pronuclei (Fig. 220). These individuals were then followed in cultures, the process of reorganization was completed, the cells regenerated perfectly, and in successful issues, normal rejuvenescence and a typical life history resulted. The crucial point, so far as the present matter is concerned was not determined, viz., from what elements were the new macro- and micronuclei derived? Did the stationary pronucleus in its "unbalanced" condition give rise to the new nuclear elements as it would have done were it an amphinucleus? Was

there a fusion prior to the degeneration of other pronuclei of the stationary pronucleus with one of the "male" pronuclei of which there may be as many as four in each conjugant? Or did the stationary pronucleus degenerate, its place being taken by one of the

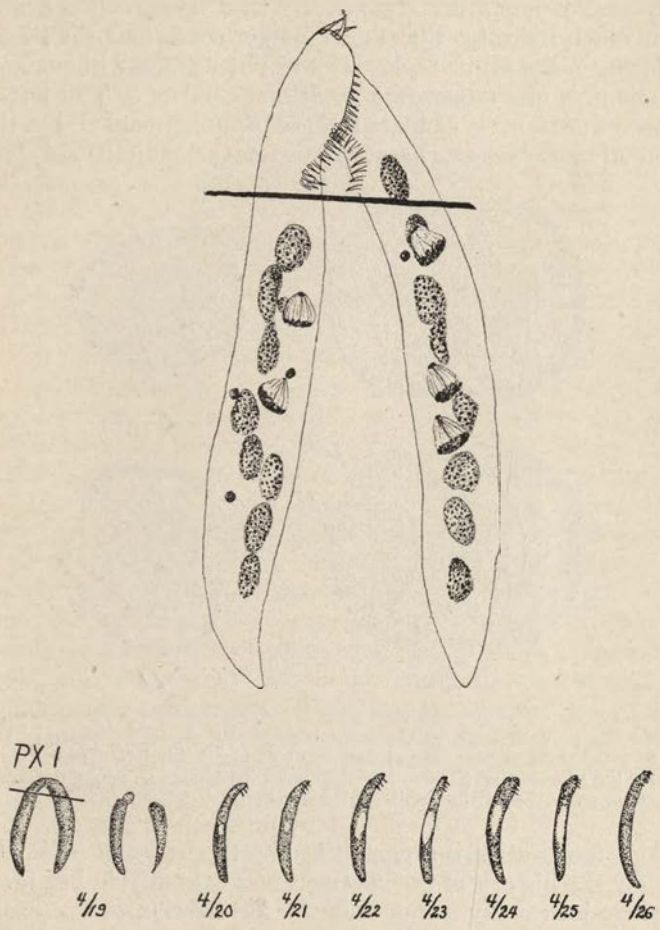

FIG. 220.—*Uroleptus mobilis*, cut during conjugation as indicated. In this case the conjugants were in the prophase stage of the first meiotic division. *PXI* history of reorganization without fertilization. (After Calkins.)

other pairs of pronuclei? Some evidence that the last alternative was the case is afforded by the fact that the conjugating pairs if cut apart at an early period in conjugation do not undergo the third division, some one of the products of the second division acting as an amphinucleus, thus realizing the condition during "endomixis,"

(*b*) **Gametic Meiosis** (Wilson, 1925).—In the preceding section instances of meiotic divisions subsequent to cell fusion were interpreted as due to stimuli mutually imparted to the conjugating individuals. For this the protoplasm must be in a mature condition, that is, with an organization considerably modified from that of the young or immature organisms. In a later section evidence is given which indicates that under proper conditions the stage is all set for a similar all or none series of phenomena without however, the stimulus of contact (see p. 540, endomixis). The latter condition termed here gametic meiosis if accompanied by the cell fusion of gametes, is characteristic of the majority of Protozoa

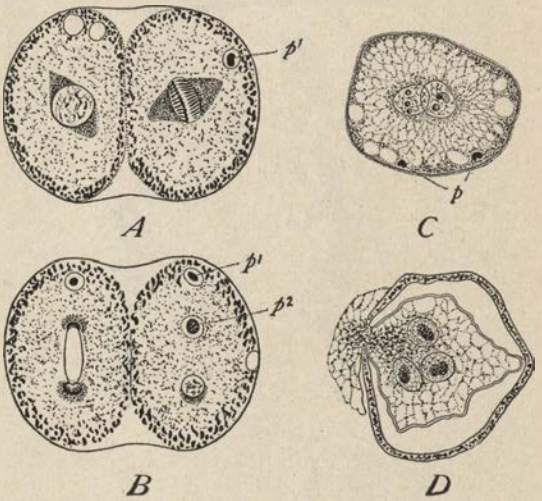

FIG. 221.—*Actinosphærium eichhornii*. *A*, two gametes ("cystospores No. 2") resulting from the division of the same mother-cell; *B*, both "polar bodies" are formed in the right gamete, the second one forming in the left gamete; *C*, the cell bodies of the gametes have fused, and the nuclei are fusing; *D*, young organism leaving cyst; p, p^1, p^2, "polar bodies." (After Hertwig.)

in which fertilization is accomplished by the fusion of cells. Unfortunately the history of the chromosomes is known in but few cases but there is scarcely a paper on the fertilization of Protozoa that does not describe two rapidly-following divisions of the nuclei prior to fusion, and these are called maturation divisions, and the resulting nuclei "reduction nuclei." In *Actinosphærium eichhornii* according to Hertwig (1898) the first evidence of the process is encystment of the adult organism and excretion of waste matters contained in the protoplasm. The nuclei are reduced in number to from 5 to 10 per cent of the original number by fusion and absorption in the protoplasm. The cell then divides into as many daughter cysts as there are nuclei and these Hertwig calls cytospores No. 1,

each of which secretes a gelatinous envelope about itself. The nucleus then divides by mitosis followed by division of the cell into two daughter cells which he calls cytospores No. 2. The nuclei of the latter undergo two successive "maturation" divisions resulting in one pronucleus and two "polar bodies" in each (Fig. 221), the latter degenerating and disappearing. The two cytospores of the second order now unite again, reforming cytospore No. 1 and fertilization is completed by fusion of the pronuclei (Fig. 221). Bělař quite recently (1922) has given a more complete description of the process in the allied form *Actinophrys sol.* The individuals draw in their pseudopodia, ordinary vegetative division of the nucleus follows, and the cell divides into two. By this division which Bělař terms the "progamous" division, the two gametes are formed and after each of them has undergone two meiotic divisions of the nuclei they reunite to form the zygote. One of them anticipates the other in these divisions and develops a pseudopodial process which the other lacks. By this process the first fusion of the two cells takes place. The original cell thus is a gamont and the fusing gametes are sister cells, one of which shows an incipient sex difference in its precocious activity and by its pseudopodium-like process. (Fig. 209, p. 501). There are 44 chromosomes in the vegetative mitoses of *Actinophrys sol* and after the progamous division the gametic nuclei swell, chromosomes arrange themselves in pairs (parasynapsis) oriented towards one pole of the nucleus. These double chromosomes shorten and ultimately form the nuclear plate of the first meiotic spindle. Here the two parts of the double chromosomes are separated and pass to the resulting nuclei each of which thus has 22 single chromosomes. A second meiotic division results in the longitudinal splitting of these 22 chromosomes so that the pronuclei and the two "polar bodies" in each gamete have 22. One of the products of each division degenerates and is absorbed in the cytoplasm, and these are compared with the polar bodies in Metazoa. The two gametes then fuse, their nuclei fuse and the zygote becomes encysted (Fig. 209). In this case the chromosome cycle is remarkably similar to that of chromosomes of the metazoan egg and sperm in their maturation divisions.

Analogous processes may take place in other types of Protozoa in which fusion of gametes occurs, but the chromosome history is known in but few cases. In Gregarinida there are several progamous divisions of the gamonts the last of which according to Mulsow's (1911) observations of *Monocystis rostrata* being a reducing division whereby the chromosomes are reduced in number from 8 to 4 (Fig. 63, p. 122).

(*c*) **Zygotic Meiosis** (Wilson).—Reduction in number of chromosomes subsequent to nuclear fusion of gametes occurs in rare

instances but the phenomenon may be more widely spread than is at present admitted. Two well authenticated cases are the coccidian *Aggregata eberthi* and the gregarine *Diplocystis schneideri*. Dobell (1915) describes 6 chromosomes in the vegetative divisions of

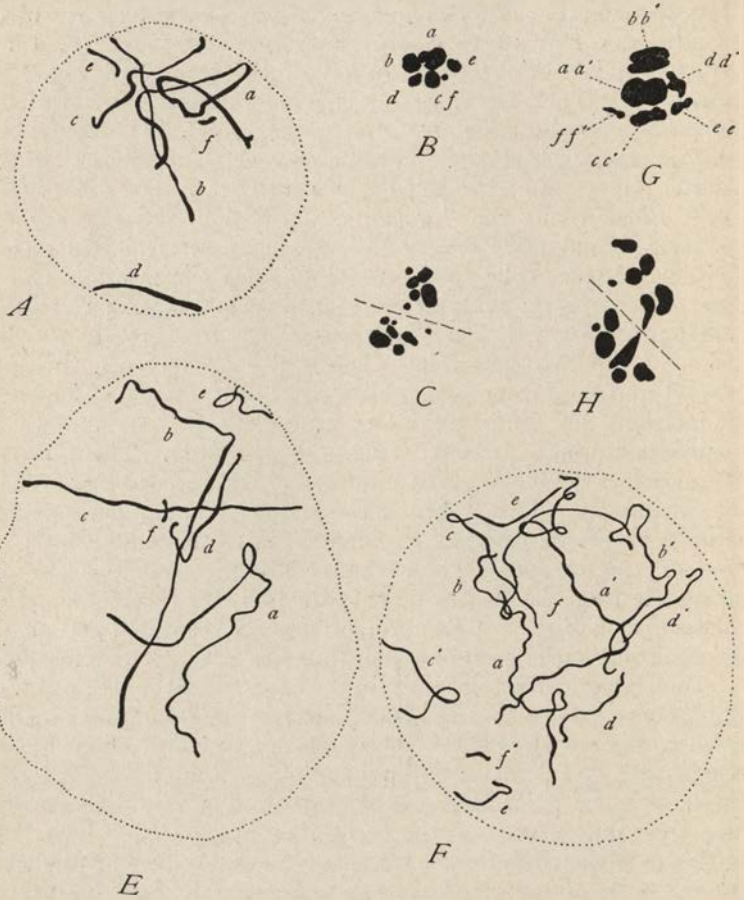

FIG. 222.—Chromosomes of *Aggregata eberthi*. Letters *a* to *f*, or *a'* to *f'* designate the haploid groups. *A*, prophase of the first division (male); *B*, nuclear plate of same; *C*, anaphase groups at first division; *E*, chromosomes in macrogamete nucleus before fertilization; *F*, chromosomes in zygote nucleus (diploid); *G*, paired chromosomes in nuclear plate of first zygote division; *H*, early anaphase groups of first zygote division, and separation of homologous haploid groups. (After Dobell and Jameson.)

Aggregata eberthi and Jameson (1915 and 1920) describes 3 in *Diplocystis schneideri* (Figs. 222, 223). These numbers remain constant in both organisms during gametogenesis, the mature gametes have the same numbers while the diploid numbers 12 and 6 are present only in the zygotes (Figs. 222 and 223). With the

first division of the zygotes the two sets of chromosomes unite in homologous pairs; in *Aggregata* 1 pair consists of long chromosomes, 1 pair is very short and 4 pairs are intermediate in length (Fig. 222). The nuclei resulting from this first metagamic division have 6 chromosomes each in *Aggregata* and 3 each in *Diplocystis* and these haploid numbers are retained throughout the vegetative cycles.

The generalization made by Dobell and Jameson to the effect that this method of reduction is probably universal among the Telosporidia is hardly justified by these two cases. Few species indeed have been studied with respect to the reduction of chromo-

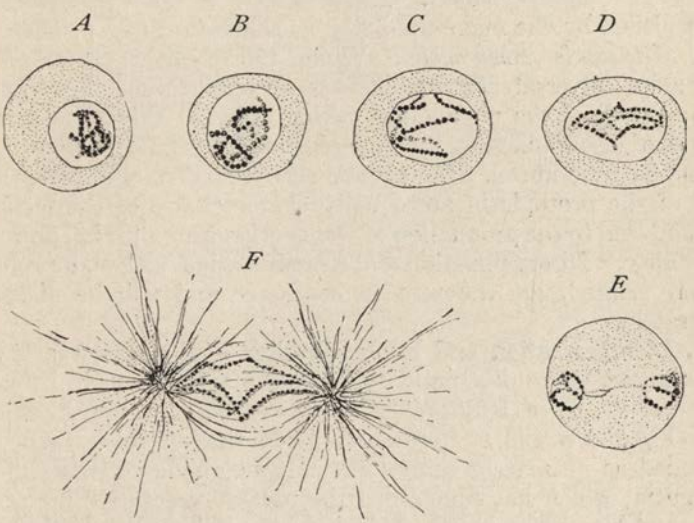

FIG. 223.—*Diplocystis schneideri*. Zygotic meiosis. *A* to *E*. nucleus of the zygote forming 6 chromosomes (the diploid number), and the first metagamic division; *F*, anaphase of the sixth progamous division preparatory to gamete formation, with 3 longitudinally split chromosomes, the haploid number. (After Jameson.)

some number and only one—*Monocystis rostrata*—by Mulsow (1911), with sufficient care as to cytological detail to be admitted, and here as stated above, reduction occurs with the final progamous division of the nuclei. Dobell and Jameson would explain this divergent case as due to confusion by Mulsow of stages of two different gregarines one with 8 the other with 4 chromosomes, but before sweeping away a difficulty in this naïve manner it would be well to reëxamine *Monocystis rostrata* in the light of the more recent work. Evidence in support of Dobell and Jameson's generalization is furnished by the fact of the frequent occurrence of an odd number of chromosomes in nuclei of different gregarines. Thus 5 chromosomes were found by Shellack (1907) in *Echinomera hispida* and

the same odd number by Léger and Duboscq (1909) in *Nina gracilis;* while 3 were found by Shellack in *Monocystis ovata* (1912). Such odd numbers are not difficult to interpret if reduction takes place at the first metagamic division but they lead to questionable hypotheses of "odd chromosomes" (Léger) "accessory chromosomes," etc., if reduction is interpreted as taking place prior to fertilization. Trouboukoff's (1914) account of reduction in the macrogamete as occurring either before, during or after fertilization in *Stenophora juli* is unintelligible under either interpretation.

Apart from Sporozoa the only evidence of zygotic meiosis in Protozoa is given by Pascher's (1916) account of Mendelian segregation in *Chlamydomonas.* This evidence is not cytological but is furnished by the make-up of the F_2 generation (see p. 577).

3. *Metagamic Phenomena.*—While the meiotic processes are probably universal accompaniments of fertilization they do not comprise all of the phenomena taking place at this period. Evidences of disorganization are apparent in the cell quite independent of the gametic nuclei. Metagamic activities involving reorganization of the protoplasm are equally characteristic of the fertilized cell and lead to the production of young organisms with full potential of vitality. Disorganization and reorganization, although probably closely related, are different in character and will be discussed separately.

B. Disorganization and Reorganization.—(*a*) **Phenomena of Disorganization.**—The destruction of the old macronucleus in Infusoria is one of the most significant of the phenomena attending conjugation (Fig. 206, p. 496). Here is an organ of the cell which is generally regarded as intimately connected with metabolic activities of the organism; which has functioned throughout vegetative life of the race and has divided with each division of the cell. Yet at conjugation the macronucleus degenerates through hypertrophy and fragmentation and the fragments are ultimately absorbed in the protoplasm. The process is fundamentally the same in all ciliates differing only in details.

If the organization of a ciliate is dependent upon the specificity of the proteins, carbohydrates, fats, salts and water which enter into its make-up then this large bulk of nucleo-proteins distributed to all parts of the cytoplasm, must bring about a markedly different matrix with which the new amphinucleus and its products are to react. Zweibaum (1922) concluded that products of metabolism during vegetative activity gradually poison the nuclear substances so that both synthetic and oxidizing activities are weakened, but at conjugation and with fragmentation of the macronucleus the contained ferments are freed from their toxic bonds, and activity is fully restored. The intake of oxygen is much greater after conjugation than before, a fact which Zweibaum (1921) interprets as

due to reorganization and the freeing of oxidases by nuclear disorganization. To this mass of nucleo-proteins is also added three-quarters (*e. g.*, *Paramecium*) to fifteen-sixteenths (*Uroleptus*) of the substance of the old micronuclei, which is likewise absorbed in the cytoplasm.

Not only is the old nuclear material broken down and distributed but, in some instances at least, the formed metaplastids of the cell are similarly destroyed and absorbed. This is well illustrated by the disappearance of the old pharyngeal basket and some of the cilia, of *Chilodon uncinatus*. (MacDougall, Fig. 106, p. 225). This is perhaps relatively unimportant at conjugation since the same thing happens at each division of the cell during vegetative life, but it is evidence in support of the view that stabile substances of the organism, substances that have accumulated with continued vegetative life are reduced to labile substances at this significant period of the life history.

In a similar manner the many nuclei of *Actinosphœrium eichhornii* (300 or more) according to Hertwig (1898) are fused together or absorbed prior to fertilization. As there must be a limit to the number that fuse (if any?) the great majority of nuclei must be absorbed in the protoplasm, for only a few (up to 20) become nuclei of gamonts (see p. 530).

In gregarines also there is a similar fragmentation of some of the nuclei leading to collections of chromidia which appear to function in the formation of sporoducts (see p. 493). In Mycetozoa and Neosporidia also some of the nuclei are destroyed in connection with the formation of accessory structures of the fruiting bodies (elaters, sporoducts, spore capsules, etc.).

The conclusion is forced upon us that this period of fertilization is marked by far-reaching changes in organization. Some of these, as in ciliates, have a prospective value for the young organisms while other are differentiations serving a useful purpose for the limited period of fertilization in organisms whose individual metabolic activities are approaching the end, and these are evidence of extreme specialization.

(*b*) **Metagamic Activities and Reorganization.**—Under this heading we include all changes which take place in the organism immediately after formation of the amphinucleus. In ciliates the fragmentation and absorption of the old macronucleus may continue for several days after union of the gametic nuclei but the further activities of the amphinucleus appear to be independent of the other happenings in the cytoplasm. These activities have to do primarily with the differentiation of the characteristic cell structures of the new organism. Thus in *Chilodon* and other Chlamydodontidæ a new oral basket is formed and some if not all of the cilia are renewed; whether or not new cirri, membranelles, and undulating membranes

are formed and the old ones absorbed, has not been fully determined by observation but this appears to be the case in *Uroleptus mobilis*.

The most important of the changes at this period have to do with the formation of the new macro- and micronuclei. The inaccurate statement is often made to the effect that the new macronucleus is formed by the metamorphosis of a micronucleus. This is strictly true only in cases of parthenogenesis. In fertilization both macro- and micronucleus are formed from products of the amphinucleus and both types of nuclei are formed by metamorphosis of such

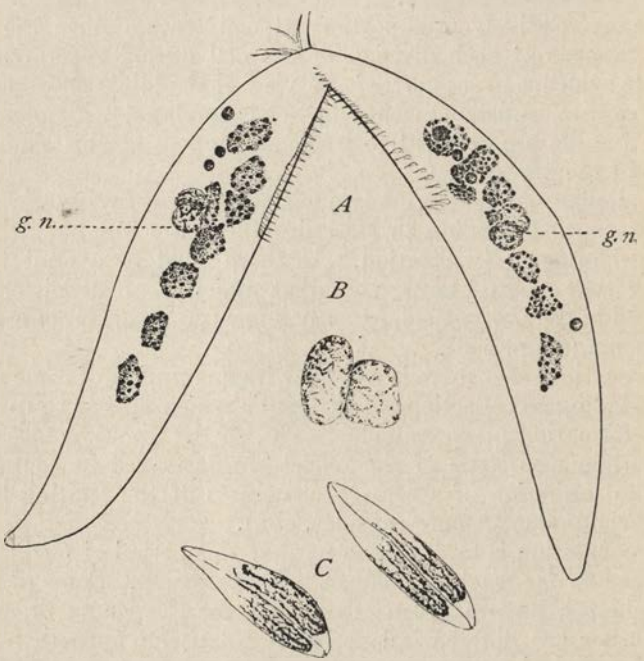

FIG. 224.—*Uroleptus mobilis;* conjugation at the stage of nuclear fusion: *g*, *n*, gametic nuclei about to fuse; *B*, same enlarged; *C*, elongation of amphinucleus shortly after fusion. (After Calkins.)

products. In the majority of cases the first metagamic division of the amphinucleus results in two equivalent nuclei. In *Uroleptus mobilis* this division occurs very soon after fusion and before complete mixture of the two pronuclei is established (Fig. 224). This is shown by the occasional finding of nuclei in which 4 of the 8 chromosomes are in the anaphase stage while the other 4 are in the metaphase (Fig. 225). The two products of this division have different fates. One of them divides again to form two nuclei which lose their vesicular character and condense into minute and homogeneous bodies, the micronuclei. The other one forms a

heteropolar spindle and divides into two unequal products the larger of which is vesicular and persists as the new macronucleus, the smaller one is spheroidal and compact and ultimately disappears by absorption (Fig. 225, 4). The young macronucleus sometimes

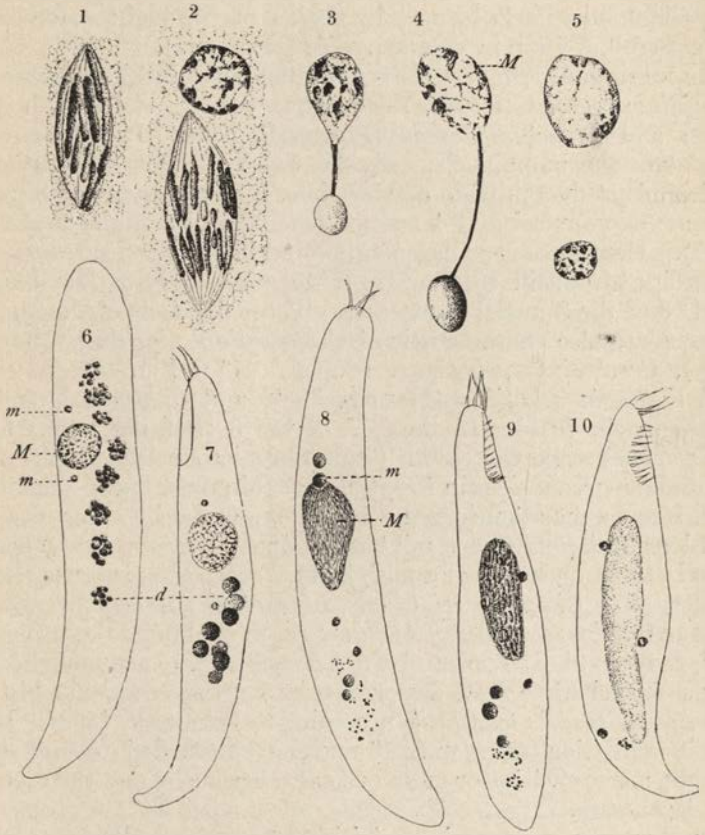

FIG. 225.—Origin of macronucleus after conjugation in *Uroleptus mobilis*. (1) first metagamic mitosis of the amphinucleus; (2) one of the progeny of this division dividing again; (3), (4), (5) telophase stages of second division of the amphinucleus resulting in a new macronucleus (above), and a degenerating nucleus (below); (6 to 10), stages in differentiation of the young macronucleus and disintegration and absorption of the old macronucleus; in (10) two new micronuclei are in mitosis preparatory to the first division of the ex-conjugant. (*M*) new macronucleus; (*m*) new micronuclei; (*d*) degenerating old macronuclei. (After Calkins.)

called the "placenta" becomes finely granular and loses its staining capacity which is not regained for a period of from three to five or more days. During this period the young macronucleus appears like a vacuole in a center of a cell and is distinctly visible in the living cell. It is small at first but grows in size from day to day

until it occupies fully two-thirds of the cell. It then condenses into a compact homogeneous ellipsoidal nucleus, invisible in the living cell, and stains intensely with chromatin dyes (Fig. 225, 10). It is now ready for the first macronuclear division and divides twice prior to division of the cell. It is perhaps significant that a similar dense ellipsoidal nucleus is formed by fusion of the eight macronuclei prior to cell division in vegetative life (see p. 221).

An essentially similar history of the amphinucleus occurs in *Colpidium colpoda* (Hoyer, 1899), *Stylonychia pustulata* (Maupas, 1889) and *Lionotus fasciola* (Prowazek, 1909). In *Paramecium caudatum* the amphinucleus divides twice without differentiation and all 4 products divide a third time, 4 of the resulting 8 nuclei become micronuclei and 4 become macronuclei (Calkins and Cull, 1907). Here there is no degeneration but in *Paramecium putrinum*, according to Doflein (1916) and in *Paramecium bursaria* (Hamburger, 1904) 3 of the 8 nuclei degenerate. Three divisions of the amphinucleus are also characteristic of *Cryptochilum nigricans* (Maupas, 1889), *Carchesium polypinum* (Popoff, 1908), *Vorticella monilata* and *Vorticella nebulifera* (Maupas, 1889) and *Ophrydium versatile* (Kaltenbach, 1915). In these, 7 of the 8 resulting nuclei form macronuclei while the eighth forms the micronucleus. All 7 fuse to form 1 macronucleus in *Cryptochilum* (Maupas) but in the others each forms a macronucleus the 7 being separated by successive cell divisions until finally each cell has 1 (Popoff, Maupas, Kaltenbach).

In *Didinium nasutum* (Prandtl, 1906), *Paramecium bursaria* (Hamburger, 1904) *Glaucoma scintillans, Leucophrys patula, Spirostomum teres* and *Stylonychia pustulata* (Maupas, 1889) differentiation occurs with the second division; 2 of the 4 nuclei become macronuclei and 2 micronuclei while none degenerates. A very exceptional history occurs in *Bursaria truncatella* according to Prowazek (1899). Here no differentiation occurs until 16 nuclei are formed; 2 to 5 of these become macronuclei; 3 or more become micronuclei and the remainder degenerate.

In Sporozoa metagamic activities take quite a different form. The majority of gregarines become gamonts which form many gametes (in *Ophryocystis* only one), which copulate within the sporocyst (Fig. 180, p. 425). The amphinucleus of each zygote divides, usually three times, to form eight products each of which becomes the nucleus of a sporozoite. In *Diplocystis schneideri* the first of these divisions results in the reduction in number of chromosomes to one-half (Jameson, 1923; see p. 533). In the Coccidia the number of metagamic divisions is still further increased. Here the zygote as well as the amphinucleus divides to form from two to many sporozoite-forming centers—the sporoblasts—each of which becomes enclosed in a special sporoblast capsule where it divides, usually only once, to form sporozoites (see p. 419). In

Aggregata eberthi as in *Diplocystis* the first division of the zygote results in halving the number of chromosomes (Dobell, 1916). The Hæmosporidia differ in that capsule-bearing sporoblasts are not formed. Here the zygote grows to large size and the amphinucleus divides repeatedly until myriads of sporozoites are formed. In these types of Protozoa, therefore, metagamic activities involve actual reproduction and reproduction here is a sequel to fertilization.

Other groups of Protozoa differ widely in their metagamic activities and some types gives unmistakable evidence of ontogenetic development. Thus zygotes of Foraminifera grow directly into the more or less complex asexual generation (microspheric). Here the amphinucleus divides repeatedly while the cell divisions are suppressed. Similarly in Phytomonadida the zygote after a resting period divides to form a colony of specific character and the metagamic divisions are associated with cellular differentiations no less regular in sequence than they are in many celled animals and plants.

Other changes of a metagamic nature have to do with the clearing up of accumulated substances in the cytoplasm. Zweibaum (1922) finds that relatively large droplets of neutral fat which are characteristic of vegetative phases of *Paramecium* are broken down prior to conjugation while smaller droplets of another type accumulate. Among these he was able to detect a larger amount of cholesterin ester than normal and a great quantity of what he interpreted as fatty acids. After conjugation these small drops disappear and neutral fats reappear. A similar accumulation of fat-like droplets and "lipoplasts" is described by Bělař (1922) in *Actinophrys sol* as characteristic of the copulating gametes and of the zygote, but the accumulation breaks down and disappears with germination of the latter. Macrogametes of Coccidia have an analogous store of cytoplasmic substances of the nature of lecithin which also disappear during metagamic activities.

There is some evidence, therefore, that specific products of metabolism accumulate in cells of Protozoa prior to fertilization and that these are utilized as are yolk substances of metazoön eggs in the early metagamic activities. Their disappearance after fertilization indicates that in this respect also, the general make-up of the cytoplasm is reorganized.

IV. PARTHENOGENESIS.

Parthenogenesis may be briefly defined as the development of an organism from an egg cell (or its equivalent, *e. g.*, a ciliate) which has not been fertilized. The phenomenon occurs spontaneously in a few animal groups and may be induced artificially in eggs from animals of widely different phyla which usually undergo fertilization before development.

The chief biological interest of parthenogenesis centers in the nuclear phenomena. Under ordinary conditions of fertilization two polar bodies are formed by the maturing egg and with their formation the number of chromosomes is reduced to one-half so that egg pronucleus and polar body nuclei are haploid. It follows, therefore, that in artificial parthenogenesis all tissue cells of the body are haploid. The same phenomenon occurs, naturally, in the development of the drone honey bee, or of the male rotifer and may be referred to hereafter as Type 1. In the great majority of parthenogenetic eggs, however, the second polar body is not formed and the nucleus remains diploid as for example in parthenogenetic aphids or female rotifers; this may be designated Type 2. A third possibility, in theory, would be cases where two polar bodies are formed which, with the pronucleus, are haploid but the egg becomes diploid by later fusion of the pronucleus with one of the polar body nuclei. This which may be called Type 3 has not been established with certainty in any metazoön but was suggested as a possibility by Boveri (1887) and described by Brauer (1893) as one type of parthenogenesis in the eggs of Artemia.

In Protozoa many cases of so-called parthenogenesis have been described some of which fall in line with one or another of the three types in Metazoa as outlined above. These phenomena may be grouped under two headings—so-called endomixis of Woodruff and Erdmann (1914) and autogamy, a widely used term in connection with Protozoa.

A. Endomixis.—Under this term Woodruff and Erdmann (1914) described "a complete periodic nuclear reorganization without cell fusion in a pedigreed race of *Paramecium*." At regular intervals of approximately thirty days they found that the old macronucleus of *Paramecium aurelia* gives rise to buds or fragments which are absorbed in the cytoplasm. There appears to be some difference in the details of macronucleus fragmentation between individuals in 1914 and more recent individuals. Thus Woodruff and Spencer (1922) find that ribbon or skein formation prior to fragmentation and characteristic of conjugation, which was very rare in 1914, had become much more common in 1921. Each of the two micronuclei divides twice, forming 8 products some of which form new micronuclei some new macronuclei. The possible combinations of nuclei and their relations are shown in Fig. 226. Later, Erdmann and Woodruff (1916) demonstrated a similar periodic reorganization at intervals of approximately sixty days in *Paramecium caudatum*. In this case the single micronucleus divides three times forming 8 nuclei 4 of which become macronuclei, 2 possibly degenerate, and 2 persist as new micronuclei.

In *Paramecium*, therefore, the first two divisions of the micronuclei in endomixis correspond to the reducing divisions in conjuga-

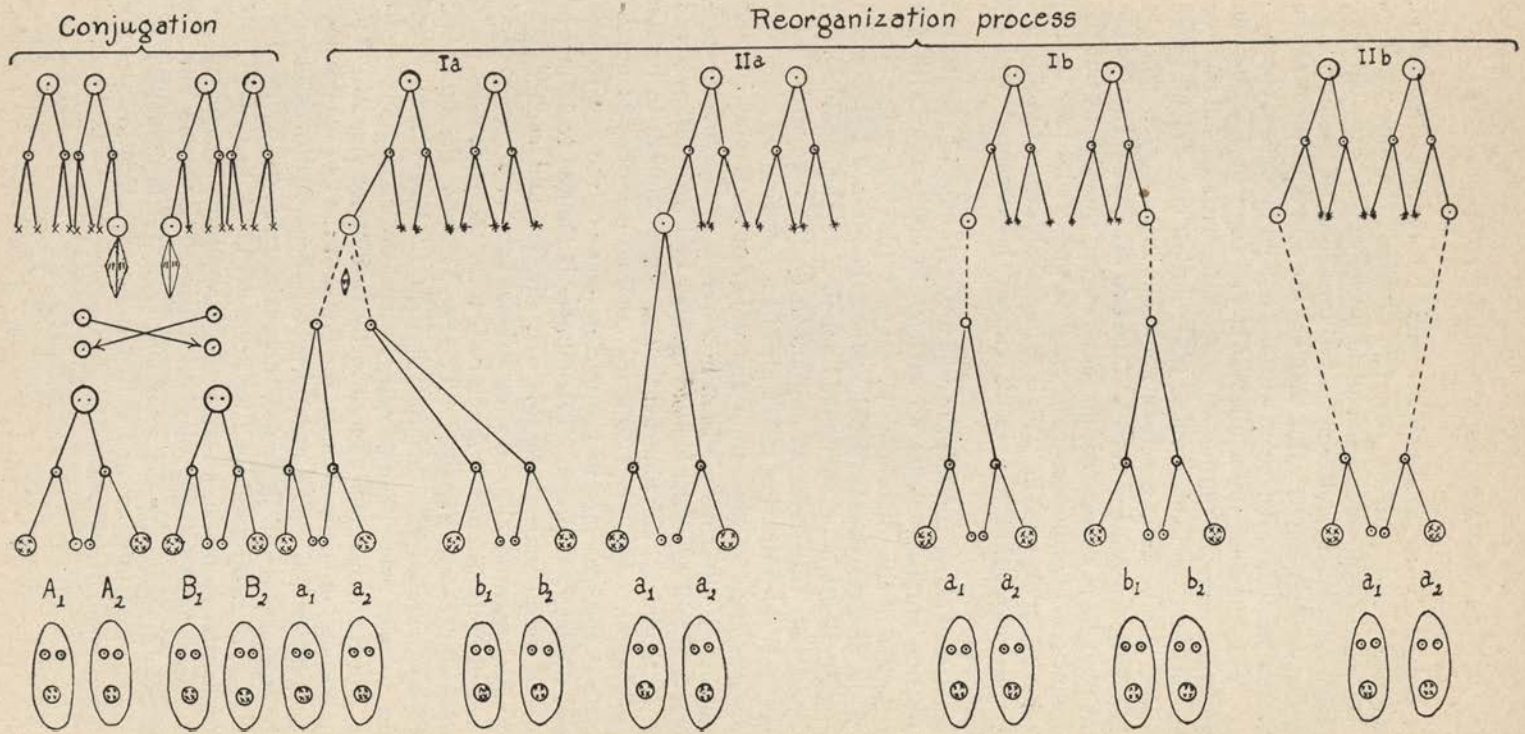

FIG. 226.—Diagram of reorganization in *Paramecium aurelia* after conjugation and during endomixis. (After Woodruff.)

tion, the third division as absent in *aurelia* but present in *caudatum*. If reduction occurs with the first two divisions the four products in endomixis are equivalent to haploid nuclei so far as the chromosomes are concerned, and correspond, therefore, to the first type of parthenogenesis above. But they are likewise equivalent to the fertilization nucleus and develop with the diploid number of chromosomes. This number, unfortunately is too large in *Paramecium* to permit of accurate counting, while in ciliates with a small number of chromosomes, endomixis takes place during encystment where cytological details have not been made out in any case. Fermor (1912) indeed, described the union of the two macronuclei and of the two micronuclei in *Stylonychia pustulata* during encystment but the account of the phenomenon is incomplete and on its face implies the fusion of diploid nuclei. This is so improbable from the chromosome standpoint that the result cannot be accepted without confirmation.

As indicated above (p. 527) the difficulty over haploid and diploid chromosome number reaches an extreme in connection with the third division of the ciliate nucleus. If reduction in number occurs during the first two meiotic divisions then the pronuclei are formed by a third division of an haploid number of chromosomes. If this division is transverse as appears to be the case with *Paramecium*, this third division might also be a reducing division, and the amphinucleus coming from the union of such nuclei would be haploid. If the third division however, is equational the pronuclei would still have the haploid number and their fusion would result in a diploid amphinucleus. The latter appears to be the correct solution. Gregory (1923) for example describes 24 dumb-bell-shaped chromosomes in the nuclear plate of the first meiotic division of *Oxytricha fallax*. This number is reduced to 12 dumb-bell-shape chromosomes with this first division and each dumb-bell divides longitudinally. The equational halves are separated at the second division and 12 dumb-bells form the equatorial plate of the third division (Fig. 227). The two halves of the dumb-bell are finally separated with this third division, 12 single chromosomes passing to each pole. The pronuclei thus have 12 single chromosomes and the amphinucleus formed by their union has 24. The interpretation here depends upon the origin of the 24 chromosomes of the first division. The meiotic process begins with a spireme which fragments into granules, approximately 48 in number. Association of these granules 2 by 2, results in 24 dumb-bells. If the number of chromosomes were 48 this would be synapsis in the usual sense. The reduced number, however, is 12 and only 24 chromosomes make up the amphinucleus. If the granules are homologous and in pairs, and if like unites with like to form the dumb-bells, then division of the 24 chromosomes of the first nuclear

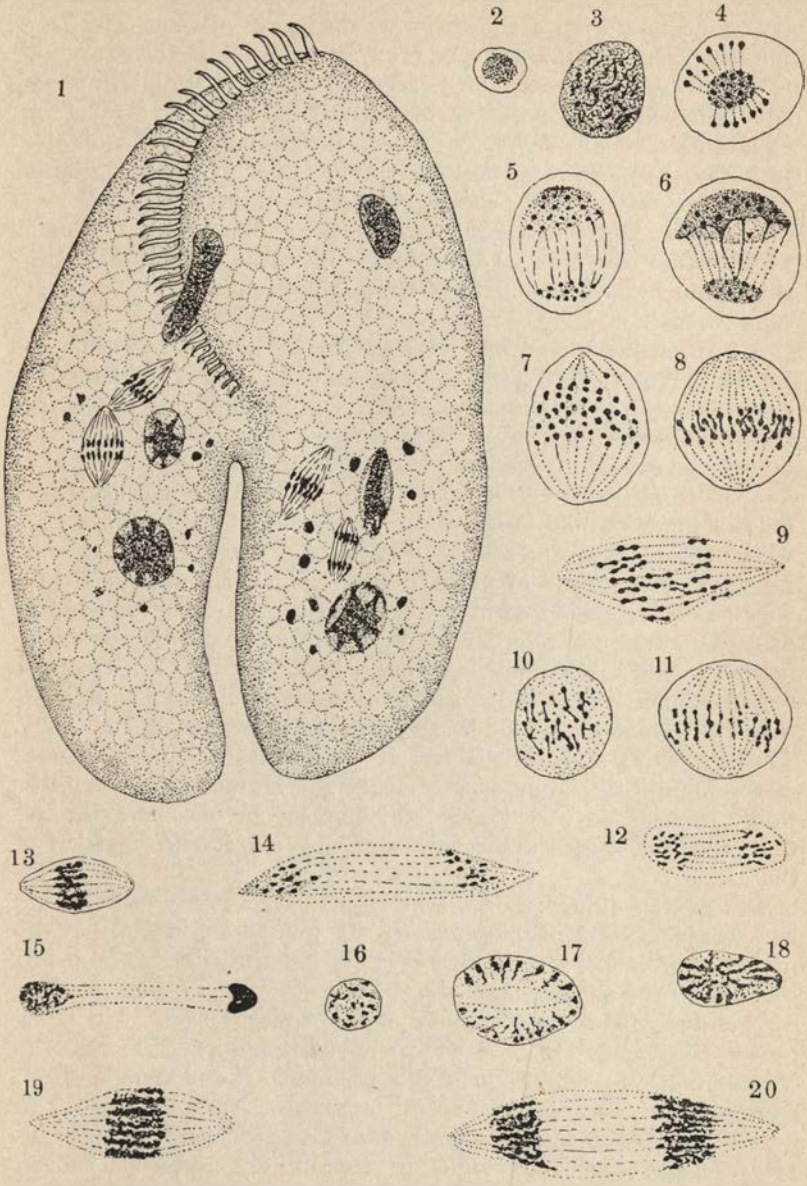

FIG. 227.—*Oxytricha fallax;* conjugation and meiosis. 2 to 9, formation and division of the first meiotic nuclear spindle and separation of the twenty-four dumb-bells into two groups of twelve dumb-bells each; 10 to 12, the second meiotic division; 13 to 15, the third division; 16, one of the pronuclei; 17 to 20, the first zygotic division. (After Gregory.)

plate in meiosis would be equivalent to equational division. The latter interpretation satisfies the conditions in other ciliates (*e. g.*, *Chilodon, Uroleptus, Didinium,* etc.), and the anomalous condition in ciliates generally may be cleared up by the assumption of two equational and one reducing division at meiosis, as against one equational and one reducing division in Metazoa. With different forms furthermore reduction may occur either in the first division as in *Oxytricha,* or in the second division as in the majority of cases on record. Dehorne (1920) escapes the difficulty by finding in *Paramecium caudatum* that there are no chromosomes at all, the single, much-looped filament of chromatin dividing transversely at each division.

A further difficulty arises with parthenogenesis. Woodruff and Erdmann regard the first two divisions of the nucleus at endomixis as equivalent to the first two divisions in conjugation. If this is true the chromosomes are presumably reduced in number by either the first or the second division and the reorganization nucleus would be haploid from which the normal number of chromosomes in endomictic animals would have to be reëstablished by division of each of the chromosomes present. In the case of *Oxytricha fallax* cited above, barring fusion of nuclei during endomixis, no evidence for which has been advanced in any ciliate (with the exception possibly of *Stylonychia pustulata,* see above), the functional nucleus would have 12 dumb-bell-shaped chromosomes. If the chromosomes remain double a race of haploid individuals would be formed. At the next endomictic period these would again be halved, and so on. This, however, is unbelievable. If on the other hand, the parts of the dumb-bell should separate then the normal diploid number would be restored with two sets of homologous chromosomes and the 48 chromosomes would be formed by the further division of the 24.

Still further difficulties are added by the merotomy experiments with conjugating *Uroleptus mobilis.* A pair in conjugation at the period of pronuclei interchange is cut across the angle as shown in Fig. 220. The angular apex thus cut off and one of the arms, are fixed and stained to determine the stage of maturation. The other arm is cultivated. Since other pronuclei usually degenerate, it is evident that only one pronucleus is present in the piece cultivated, and this one contains the haploid number of chromosomes. The possibility remains open, however, that this pronucleus may unite with a sister pronucleus formed by sister nuclei, and which do not degenerate. In this case it would be parthenogenesis of the third type above. When such cutting experiments are successful the resultant organisms regenerate perfectly and undergo typical life histories and each individual has the normal number of chromosomes.

The most probable interpretation appears to be that the diploid number of chromosomes is restored by chromosome division. In endomictic animals chromosomes become homozygous. But after 50 or more generations of close in-breeding by conjugation the chromosomes must all have become similarly homozygous and a matter of no qualitative difference whether one set divides, or receives an homologous set in amphimixis. The 4 chromosomes of *Uroleptus* are probably qualitatively different and a full set are probably needed for complete development of the individual. There is always a possibility of imperfect segregation, resulting in failure to reorganize on the part of the ex-conjugant. This possibility is certainly not lessened by complete homozygosity and may be significant in connection with the increased percentage of deaths after conjugation which is now apparent in the cultures.

The conclusion follows that so far as chromosomes are concerned, endomixis and amphimixis after prolonged in-breeding as in *Uroleptus* are similar in results. The cellular processes of reorganization are identical in both and Woodruff is quite right in stating that amphimixis is unnecessary for continued life of a ciliate. In respect to vitality, endomixis and amphimixis are equivalent and so long as one or the other occurs, continued vitality is possible. Furthermore it may be argued that if an equivalent reorganization is accomplished in any other way then neither endomixis nor amphimixis by conjugation is necessary. Evidence of this third possibility is furnished by observations on *Paramecium calkinsi* (Spencer, 1925), by *Actinophrys sol* (Bělař, 1922), by *Eudorina elegans* (Hartmann, 1921) and by the animal flagellates. If this is a correct interpretation then there is a possibility of harmonizing the many conflicting results and views advanced in relation to the much discussed problem of indefinitely-continued vitality.

B. Autogamy.—Autogamy, or self-fertilization in Protozoa is a logical sequence of endogamy. If a gamont of *Actinophrys sol* should not divide to form gametes which later fuse (see above, p. 500), and if the gamont's nucleus should divide and the two products should undergo meiosis, and the two pronuclei should then unite, all in the same one cell, then the process would be called autogamy. Or if pronuclei from the same individual ciliate should unite, it would be autogamy. In short autogamy is the realization of Type 3 of parthenogenesis above.

The phenomena which have been described and interpreted as autogamy, particularly as they occur in parasitic forms, are rather cautiously interpreted today and many careful observers, perhaps too careful, are inclined to regard the earlier descriptions of autogamy as dealing with degeneration phenomena rather than with normal vital activities. A classical example of such earlier work is the description of autogamy in *Endamœba coli* as given by Schaudinn

(1903) and confirmed in essence by Wenyon (1907) in *Endamœba muris* (Fig. 228). The organisms encyst after a period in the host's intestine; the nucleus of the encysted cell divides (*A*, *B*, *C*,) and the cell body indicates an attempt to divide into two parts. The protoplasmic connections between these two cytoplasmic parts are never lost so that subsequent processes take place in a binu-

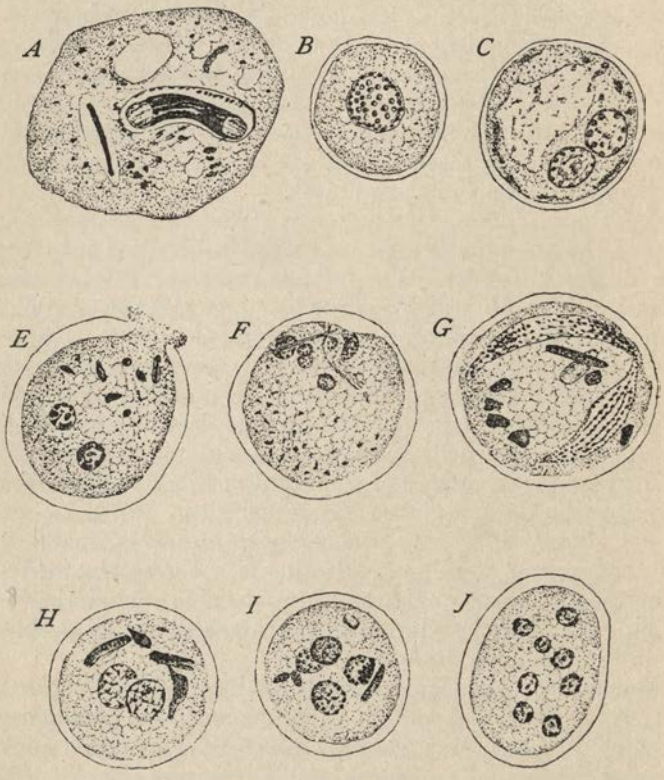

FIG. 228.—*Endamœba muris*. *A*, ordinary individual prior to encystment, in division. *B*, an autogamous cyst; *C*, division of nucleus and vacuolization of cell; these nuclei then break up into chromidia; *E*, reformation of two nuclei from chromidia; *F*, *G*, two nuclei and so-called reduction bodies remain in the cyst, the former now divide (*G*) to form 4 nuclei which unite 2 and 2; these nuclei now divide to form 4 and the 4 give rise to 8 (*J*). (After Wenyon.)

cleated cell. The nuclei next fragment, forming idiochromidia from which two smaller nuclei (*D*, *E*) are formed by segregation of the scattered granules. Each nucleus then divides twice, one-half at each division forming nuclei which degenerate in the cell ("reduction" nuclei). The other halves form two fertilization nuclei each of which divides again, this time with the long axes of the spindles

parallel with each other and the final daughter nuclei fuse 2 by 2. The cleft in the cell disappears and an encysted Amœba results with two amphinuclei. Each of these nuclei divides twice and eight spores are formed about the resulting eight nuclei (Fig. 228, *I*, *J*).

Autogamy appears to be characteristic of the Neosporidia among the Sporozoa and the processes are fairly uniform in Myxosporidia, Microsporidia and Actinomyxida. Multinucleate cells are typical of the nutritive or vegetative stage and in some cases the nuclei are dimorphic. Spores are formed endogenously and during the continued vegetative activity of the organism. The process was well described by Schröder (1907) for *Sphæromyxa sabrazesi*, a parasite of the sea horse, where the multinucleate amœboid body of the parasite contains two kinds of nuclei distinguishable by size and structure. Within the protoplasmic body small areas become differentiated from the surrounding cytoplasm. These areas, characteristic of the Myxosporidia, each contain 2 nuclei, 1 of each kind (Fig. 229, *K–Q*). With the development of the pansporoblast, each nucleus divides in such order that 7 daughter nuclei finally result from each, the 14 nuclei behaving as follows: 2 are destined to degenerate as "reduction nuclei;" 4 become the centers of capsule and shell formation; 4 become centers of polar capsule formation; and 4 remain as germinal nuclei. The protoplasm of the pansporoblast divides into two halves (*M*) the sporoblasts, and each contains 6 of the nuclei, while the 2 degenerating nuclei remain outside. The 6 nuclei are thus differentiated into somatic and germinal nuclei 4 in each case going into somatic differentiations of the spores (shells, polar capsules and threads) and 2 presumably 1 of each of the original 2 kinds, remain as pronuclei (*N*, *O*, *P*).

Many different observers have noted this binucleated stage of the young spore, and the problem of fertilization in Myxosporidia appears to be bound up with their further fate. Schröder believes that they unite later and so complete the fertilization, a belief which he was able to prove in a later publication (1910). Keysselitz (1908) working on *Myxobolus pfeifferi*, likewise believed in the union of an analogous pair of nuclei during either the final stage of development of the spore or in the young animal immediately after leaving the spore case (Fig. 229, *A–J*). Davis (1916) observed the union of such nuclei in *Sphærophora dimorpha* but was somewhat skeptical of his own observations, but Erdmann (1911 and 1917) confirmed Schröder in actually observing the fusion. Awerinzew (1909) on the other hand, working with *Ceratomyxa drepanopsettæ*, believed that fusion or fertilization does not occur in the spore stage but after the initial development of the young animal (see also Kudo, 1924). When the latter has reached the stage with 4 nuclei, 2 of the nuclei become trophic while the other 2 become

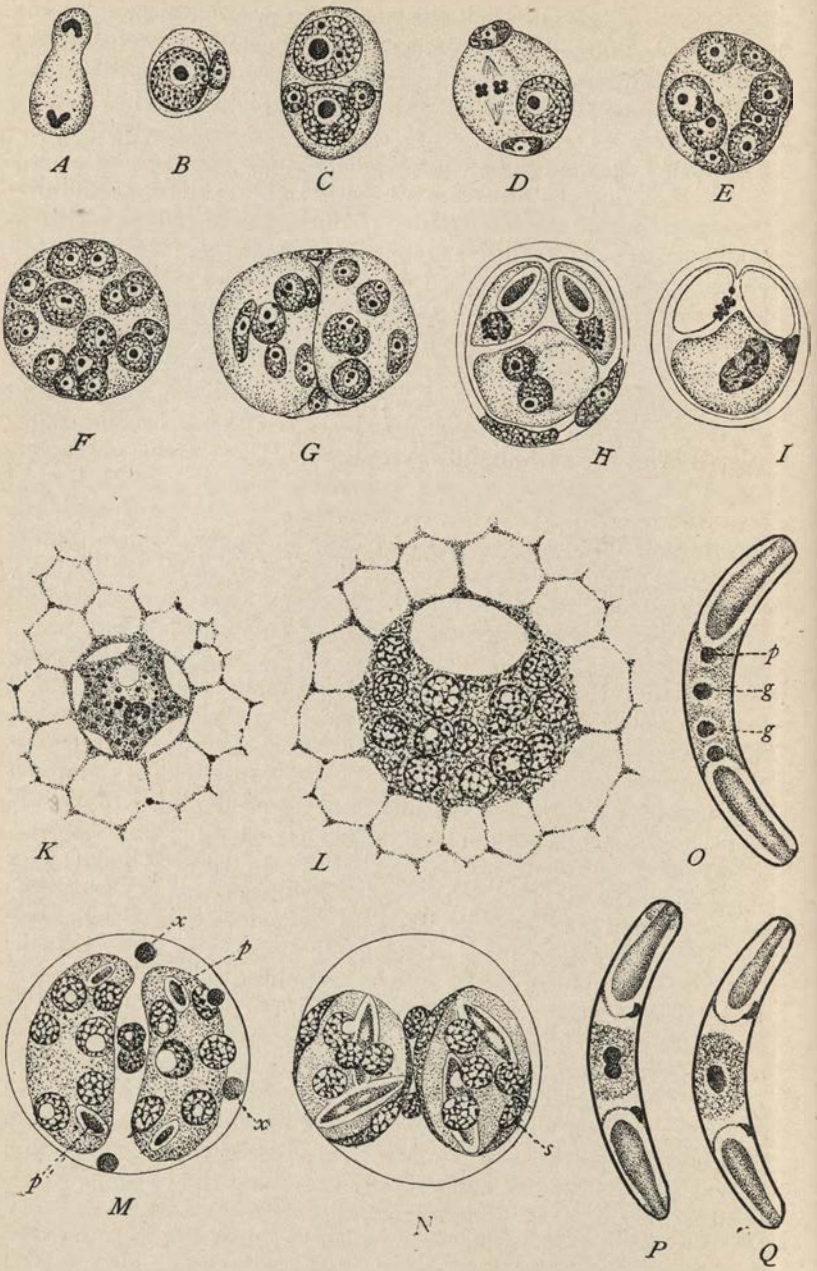

FIG. 229.—*Myxobolus pfeifferi* (*A* to *I*) and *Sphæromyxa sabrazesi* (*K* to *Q*). See text. (After Keysselitz and Schröder.)

germinal giving rise by division to "microgametes" and macrogametes which fuse after "reduction." Mavor (1916) working with an allied species (*Ceratomyxa acadiensis*) found uninucleate young forms which, upon the first division of the nucleus, give rise to dimorphic nuclei as described by Awerinzew. The fusion of "gametes" which Awerinzew described was confirmed in part by Keysselitz (1908) in connection with *Myxobolus pfeifferi.* Here the pansporoblasts which Keysselitz names the "propagation" cells,

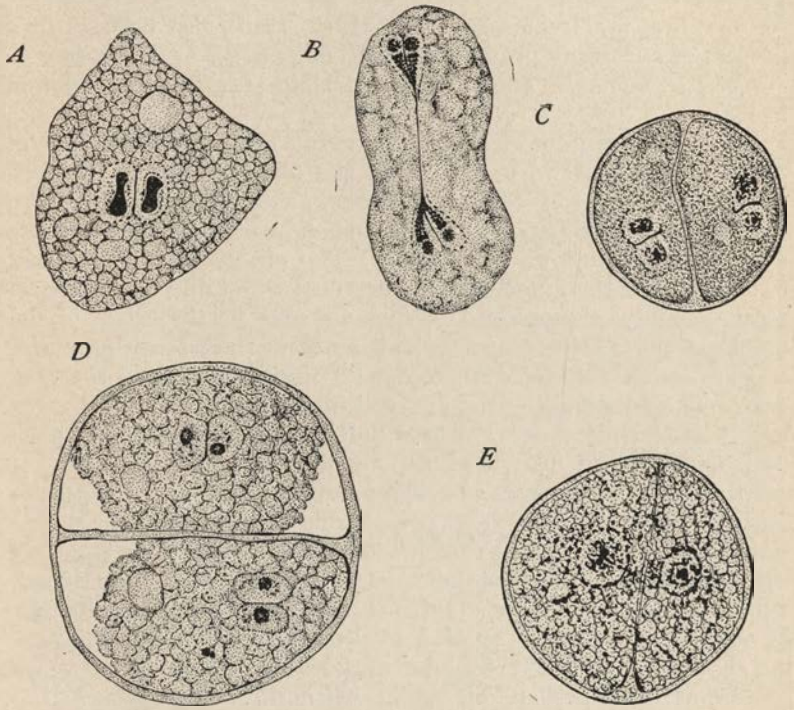

FIG. 230.—*Amœba diploidea.* The ordinary vegetative individual has two nuclei which divide independently at cell division. With encystment these nuclei form spindles (*B*) and the cells divide (*C, D*); the two pairs of nuclei then unite, forming two fusion nuclei after which the cell bodies reunite, thus forming the vegetative binucleated cell. (After Hartmann and Nägler.)

arise in the protoplasm of the adult organisms in the same manner as in other Myxosporidia, but the nuclei, and with them the cell body of the germinal area, divide (Fig. 229, *A. B, C*). The propagative cells later unite 2 by 2 and are at first separated by a thin cell wall, which later disappears. Within this united mass the nuclei divide until there are 14 as in *Sphœromyxa.* Such cases of fusion are interpreted by Erdmann (1917) as plastogamous in character.

These observations indicate that fertilization in Myxosporidia belong in the group of autogamous phenomena. In the closely related Microsporidia there is considerable difference of opinion in connection with the time and place of fertilization if it occurs at all. Stempell (1902, 1904, 1909) and Fantham and Porter (1912), give evidence to indicate that union of nuclei occurs as in Myxosporidia and after the spore leaves its capsule. Mercier (1909), Swarczewsky (1914) and others believe that the formation of heterogametes occurs prior to sporulation as described by Awerinzew for *Ceratomyxa;* Debaisieux (1913, 1915, 1916) also believes in a process of autogamy prior to sporulation in *Glugea danilewskyi, G. mulleri, G. anomala,* and in microsporidian parasites of Simulium larvæ.

Similarly a process of autogamy occurs prior to sporulation in Actinomyxida. Here, according to the observations of Caullery and Mesnil (1905) on *Sphæractinomyxon,* the youngest stages are found as intestinal parasites of the tubificid worm Clitellio, and are either uninucleated or binucleated. The observers were inclined to believe that the uninucleated stage comes first and that it represents, possibly, a sporozoite. Whatever may be the origin of the binucleated form, the 2 nuclei divide and 2 of the 4 resulting nuclei become somatic nuclei connected with the formation of the cyst wall. The remaining nuclei and cell body now divide until there are 16 independent cells. These unite 2 by 2, fertilization thus occurring endogamously, and 8 spores are finally formed.

In many of these cases so-called reduction nuclei have been described as indicating processes comparable with chromosome reduction in meiosis. Up to the present time, however, while well-marked chromosomes of definite number have been described by Georgewitsch (1915) and by Davis (1916) there is no evidence of reduction in number either before or after nuclear fusion. Erdmann (1917) has shown that so-called reduction nuclei inside the spore are masses of chromatin or perhaps glycogen, which serve a purpose in the formation of the spore membrane. The extremely minute size of the nuclei and the technical difficulties, make the general problem very difficult to solve in Telosporidia and at the present time there is little prospect of an early solution.

From the foregoing review it is apparent that the changes of a cumulative character are taking place during the vegetative activities in all types of organization. Such changes are manifested by structural or functional peculiarities at different stages, the most marked of which are at periods of maturity and old age. Some of these are peculiar to certain types only, *e. g.,* the old age structural differentiations of Mycetozoa and Sporozoa. Others, particularly those occurring at maturity, are more universal but differ in degree in different cases, the least evident being those of hologametes and

conjugating Infusoria, and the most evident are those in which complete anisogamy occurs. One widely spread effect of such differentiation is the phenomenon of meiosis or reduction in the number of chromosomes. This also occurs at various periods, furnishing a basis for the categories of conjugant meiosis, gametic meiosis and zygotic meiosis.

Whatever may be the interpretation of the phenomenon, the fact is obvious that all products of fertilization are labile, active organisms quite different in character from the conjugants, hologametes, or gametes which participated in their production. Apparently the same protoplasm, however, is continuous from the old to the young, and during transition certain processes, here described as disorganization and reorganization, have taken place. These processes, as I believe, are responsible for the renewal of vitality and for the inauguration of a new life cycle in a new organism, evidence for which is given in the following chapter.

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CHAPTER XII.

EFFECTS OF REORGANIZATION AND THE ORIGIN OF VARIATIONS IN THE PROTOZOA.

In the preceding chapters we have developed the ideas that life is organization; that vitality is the sum-total of actions, reactions and interactions between and amongst the aggregate of substances which make up protoplasm; that minute differences in the aggregate of substances constitute differences in organization; that no two organizations are identical; that with continued metabolism the protoplasm of a given individual undergoes changes in organization which are gradual but progressive; that such changes may be manifested by structural differentiations and by physiological activities which are characteristic of certain periods in the life cycle; and that progressive differentiation leads to a condition of protoplasmic stability such that metabolic activities weaken or cease altogether.

We have no desire to belittle or ignore the fact that observations are not all in accord with the conclusions outlined above or to underestimate the significance of data which apparently do not agree with them. We are attempting however, to formulate a conception of organization and vitality which will embrace as large a field of observational results as possible and to give a rational interpretation of them. An important part of such an interpretation is concerned with the effects of fertilization and parthenogenesis which are considered in the present chapter.

1. EFFECTS OF REORGANIZATION ON VITALITY.

If our fundamental thesis that continued metabolism leads to functional weakening and ultimate cessation of vitality is correct it follows that for continued life some reconstructive or reorganizing operation is necessary. The phenomena attending cell division, together with experimental evidence (see Chapter V) indicate that such reorganization may occur with each division of the cell, and that vitality of the protoplasm immediately after division is normally unhampered by accumulated products of activity in the form of metaplastids or of substances which are becoming inert. The deep-seated changes in organization which accompany fertilization and parthenogenesis have a similar but an even more profound effect for the protoplasm is entirely made over and new cell organs are

present for activity in a renewed cytoplasmic body, the aggregate resulting in a new organization and new vitality.

"Conjugation is a physiological necessity for maintenance of the race" (Hartmann, 1921; p. 114). This indeed is one of the oldest views as to the effect of conjugation of the ciliates. It is unfortunate perhaps that the phenomena involved became labeled with fanciful terms signifying renewal of youth (Verjungung of Bütschli, 1876, Rejuvenescence of Maupas, 1889), terms which many hard-headed biologists find it difficult to accept. It might or might not have made some difference if the phenomena had been interpreted as a series of reactions whereby protoplasmic impedimenta are removed leaving a renovated organism and a possibility of unhampered vitality. It is in this sense that the term rejuvenescence is used in these pages.

Another interpretation of the phenomena, however, was early given in connection with theoretical biology. The union of two individuals in conjugation or in fertilization generally, involves the fusion of two organizations represented either by nuclei alone as in conjugation, or by nuclei and cell bodies as in merogamy. The term amphimixis (Weismann) was applied to this phenomenon and its significance was interpreted as a means of inaugurating variations which would turn out to be useful or not in the grilling process of natural selection.

Of the two interpretations the former appears to be the more comprehensive and fundamental since it deals with vitality and applies not only to phenomena of fertilization but to effects of parthenogenesis as well, and may be still further extended to include the effects of periodic reproduction by cell division. The general truth of the latter interpretation is undeniable and has been repeatedly confirmed in experimental zoölogy, but we avoid the stigma of teleology by assuming that amphimixis arose in connection with the satisfying of some fundamental protoplasmic need. In other words and on this supposition, gametes were developed not as a means of ensuring amphimixis but as a result of vital activities and changes in organization which rendered them unable to continue metabolic activities without fusion.

It would seem that the fundamental truth of this generalization requires no argument insofar as it concerns merogamy. The fertilized egg cell is a new organism with a new potential of vitality having the possibility of development with differentiations leading to the adult organism. It is the beginning of a new life cycle for which the stimulus to development is furnished by the sperm cell. The facts of parthenogenesis however, show that this potential is in the substance of the egg itself and that it, without participation of the sperm cell, may likewise be the beginning of a life cycle. The egg cell furthermore does not have the same organization as

did the primordial germ cell, or endothelial cell from which it came. Reorganization of the protoplasm of that endothelial cell has taken place in its metamorphosis to an egg cell and is brought about by the often-described process of ovogenesis and maturation. In this phenomenon of endothelial cell metamorphosis we find the homologue in Metazoa of the reorganization processes of the Protozoa.

The nearest approach to the metazoön egg and spermatozoön condition amongst animal Protozoa is the group Coccidiomorpha amongst Sporozoa. Here, no less than in Metazoa, the fertilized egg is the beginning of a new life cycle, or by metagamic divisions, gives rise to sporozoites each of which is the beginning of an independent life cycle with its characteristic phases and differentiations. Few biologists would question the application to Sporozoa of the term life cycle, and yet no single individual sporozoön has ever been followed through the sequence of changes from fertilization to fertilization. This cyclical history of Sporozoa is forgotten by those like Woodruff who speak of a life cycle in Protozoa as a myth. They have in mind only the ciliated Infusoria and the phenomena of conjugation; indeed the controversy over the effects of fertilization in Protozoa has been limited almost exclusively to the Infusoria.

Actual experiments to test the effects of conjugation on vitality of the Infusoria have been few in number the majority of investigators stopping with experiments to determine the need of conjugation, *i. e.*, whether or not vitality as measured by the division-rate actually undergoes a diminution to a point where death ensues if fertilization fails (see Chapter X). Jennings (1921) has pointed out that Maupas himself never claimed that the power to reproduce is restored by conjugation although his experiments did lead him to the conclusion that ciliates undergo senile degeneration and natural death. This inconsistency on Maupas' part requires some explanation here for it is usually overlooked. His general conclusion is carried in the statement: "In regard to Infusoria my culture experiments have demonstrated that these Protozoa do not escape the general law of senescence" (1888, p. 273). From this conclusion we would naturally infer that senescence means a weakening of the general physiological processes including the power to reproduce by division. But Maupas apparently had no such conception of senescence for he adds: "The power of multiplication follows no such diminishing and parallel course. It is maintained almost intact even a long time after the other functions, and the entire organism, are shown to be greatly reduced by senile degeneration" (1888, p. 273).

The inconsistencies in Maupas' conclusions have been pointed out in another place (Calkins, 1923); it is sufficient here to state that exact data in the form of daily records of divisions were kept

by Maupas for only three series of individuals, and data for only one series (*Stylonychia pustulata*) were published in full. The graph shown in Fig. 231 was constructed from these published data and it certainly appears to bear out his conclusion concerning

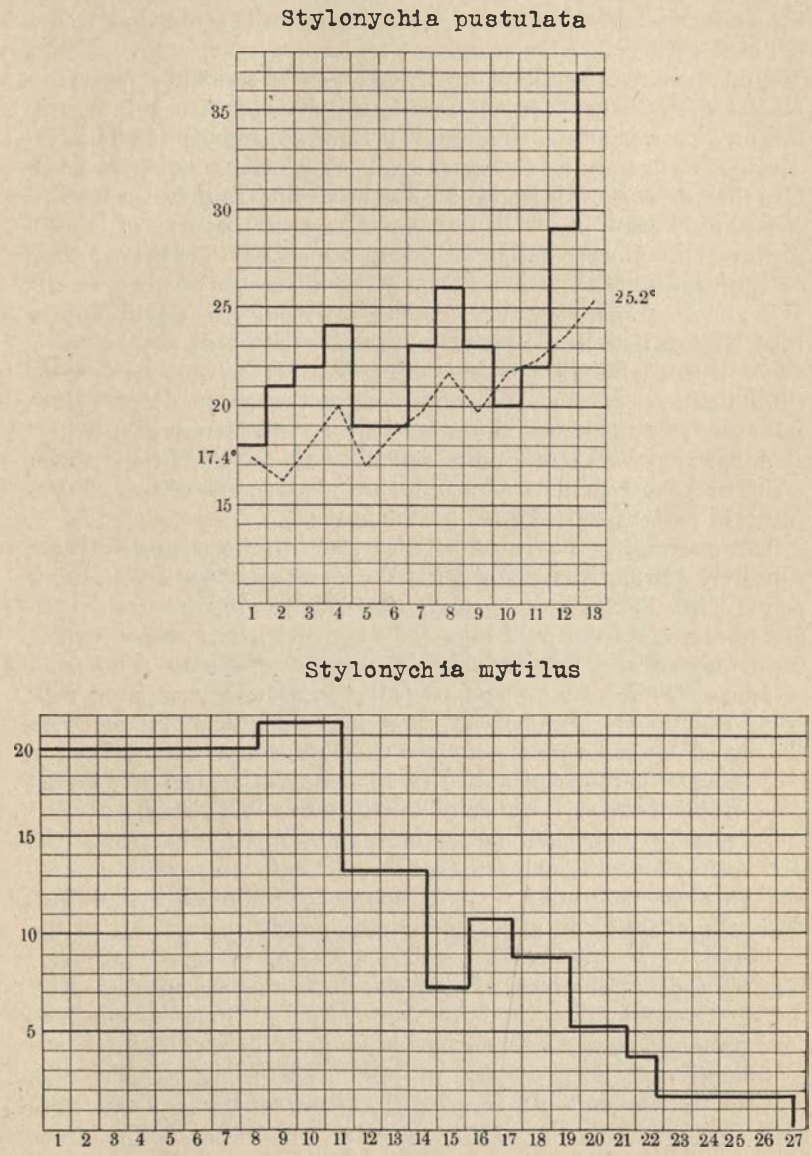

FIG. 231.—Vitality graphs of *Stylonychia pustulata* and *S. mytilus* from records by Maupas.

multiplication. For another series, however (*Stylonychia mytilus*) data were given for a different purpose and from these the graph shown in Fig. 231 (below) was constructed. From this graph it is apparent that his conclusions regarding multiplication and vitality do not agree with his records. Maupas' experimental evidence in connection with vitality after conjugation thus counts for very little either for or against rejuvenescence.

A much more carefully planned and executed series of experiments to test the effect of conjugation on the division-rate were carried out on *Paramecium* by Jennings (1913). He found: (1) That ex-conjugants in only a few exceptional cases have a higher division-rate than do non-conjugants of the same strain; (2) that conjugation causes a decrease in division-rate of the great majority of ex-conjugants; (3) that conjugation causes a high mortality among ex-conjugants; (4) that it causes a marked increase of weak, sickly, and abnormal individuals. From these results it would appear that conjugation is a highly unprofitable habit of the Infusoria which if freely indulged in by *Paramecium* would soon lead to the extermination of the race. The annual crop of *Paramecium*, however, remains about the same and we are forced to interpret Jennings' results as due more probably to the conditions under which the experiments were carried on than to the effects of conjugation (see *infra* p. 578 and Calkins, 1923).

The question of increased vitality after conjugation receives a definitely affirmative answer with Woodruff and Spencer's experiments with *Spathidium spathula* (1924). Conjugation tests furnished material from pure lines for conjugation and ex-conjugants were isolated and followed out in isolation cultures. The daily division-rates for parent and offspring series were compared with great exactness. Ninety-four different ex-conjugant series were thus available for comparison with their respective parental series. Of these the parent series died in 15 cases during the first fifteen days of life of the ex-conjugants but the latter "all actually divided more rapidly than their respective parents" (p. 187) during the periods in which the parents were alive. In 67 cases both parents and offspring continued to live and divide for more than fifteen days, the offspring in all cases dividing more frequently than the parents. Eighty-two cases therefore out of 94 ex-conjugant series showed a definitely marked increase in vitality as measured by the division-rate, as a result of conjugation: "it is evident that conjugation directly induces an immediate acceleration of the reproductive activity" (1924, p. 188). The same conclusion is reached for the full life history of ex-conjugants in comparison with the remaining life of the parental series after conjugations have occurred. "Since conjugation is the sole variable involved in ex-conjugant and parental cultures it is evident that conjugation

directly induces not only an immediate acceleration of reproduction but also an acceleration which persists at least as long as the life of the parental cultures. These results are in opposition to all results which indicate that conjugation is devoid of a profound physiological stimulation of the metabolic activities of the cell expressed in reproduction" (loc. cit., p. 189). Thus in *Spathidium spathula* not only are the division-rates of ex-conjugants higher than those of the parental strains but the ex-conjugants actually outlive the parent protoplasm, hence the authors further conclude: "Conjugation typically has a high survival value in the life of the organism" (p. 196).

It is significant that Woodruff and Spencer studiously avoid use of the term "rejuvenescence" in their work. They speak of an increased division-rate of ex-conjugants and of the "survival value" of conjugation but not of renewal of vitality. As these are the two essential factors which characterize the phenomena of rejuvenescence we are justified in including Woodruff among the proponents of rejuvenescence. The two factors were discussed in an earlier analysis of rejuvenescence (Calkins, 1920) in which it was pointed out that the division-rate expresses the "intensity" of vitality and the length of life in division days the "endurance;" the latter is evidently the same as Woodruff and Spencer's "survival value."

The experimental work on *Spathidium spathula* was a confirmation of the work on *Uroleptus mobilis* which was begun in 1917, and is still under way. A single ex-conjugant was the progenitor of all the material that has formed the subject of the investigation. The method employed throughout was the usual isolation culture method (see p. 469). In the following account of the experiments the term "series" always means an ex-conjugant with the progeny formed from it by division; the progeny being represented by five pure lines which are continued by isolation cultures until vitality is exhausted and death ensues. Conjugation tests at regular intervals provide material for filial series.[1] Up to January 1, 1925, 125 different series had been studied; 116 of them had followed the usual history and had died out and 9 series were under culture. The last of these 9 series represents the F 29 generation of successive conjugations since the original ex-conjugant was isolated. Abundant statistical data have accumulated during these seven years and these furnish valuable evidence in favor of the theory of rejuvenescence. The bearing of these data on the following topics may be briefly summarized: (1) Renewal of vitality as a result of conjugation; (2) intensity of vitality and extent of renewal; (3) effect of parents' age and vitality upon vitality of offspring; (4) evidences

[1] In the earlier publications on *Uroleptus* these series were designated by letters as Series A, Series C, etc., but with the exhaustion of the alphabet the letters have been replaced by serial numbers.

of change in nature of rejuvenescence in later series; (5) exhaustion of a strain through continued old-age breeding; (6) strengthening a weakened strain by early conjugations.

1. **Renewal of Vitality as a Result of Conjugation.**—In Chapter X it was shown that the life cycle of an ex-conjugant of *Uroleptus mobilis* begins with high vitality; this gradually weakens during a period of from nine to twelve months and ends with death of the last individual representing that protoplasm if reorganization by fertilization or parthenogenesis has been prevented. A full pedigree of the latest series (128) is illustrated by the graph shown in Fig.

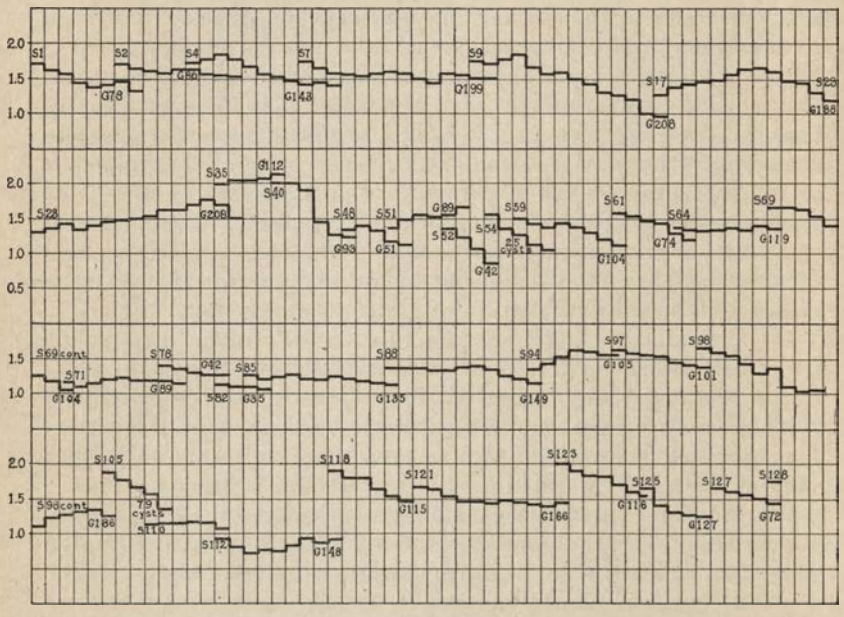

FIG. 232.—Condensed vitality graphs showing the descent of *Uroleptus mobilis* from November, 1917 to date. S = series; G = generation age of parents.

232. Conjugation between the progeny of an ex-conjugant occurs whenever a conjugation test is made after the series is mature (see p. 490). An ex-conjugant from such a mating has a higher vitality as expressed by the division-rate than the individuals of the parent series which had not conjugated. The test for this is shown by a comparison of the division-rate of the parent protoplasm which has not conjugated with the division-rate of the protoplasm that had conjugated, both protoplasms running simultaneously and under identical conditions in isolation cultures. If such conjugations occur early in the life history of the parent series both parent and offspring run simultaneously for some months;

if late in the life history of the parent the offspring series outlives the parent, in some cases for many months. An arbitrary test of the difference in vitality of parent and offspring is furnished by a comparison of the division-rate of the ex-conjugant for its first sixty days of life with the division-rate of the parent during the same calendar sixty days. The difference between the two rates indicates the difference in intensity of vitality between parent and offspring. In the accompanying synoptic table data are listed for all series to date including series number, relative vitality (column 2), number of generations attained (column 3), number of division days (column 4), parent series (column 5), age of parent series at time of conjugation (column 6); number of divisions of parent subsequent to conjugation (column 7); intensity of vitality of parent and offspring and differences between these intensities (columns 8, 9 and 10). The division-rates represent the numbers of divisions which any individual of a series would undergo in ten days.

The last column of the table on pages 560, 561 and 562 gives an emphatic affirmative to the question. Does conjugation effect a renewal of vitality?

2. **Intensity of Vitality and Extent of Renewal.**—An important matter which is usually overlooked in experiments of this nature is the intensity of vitality of the parent protoplasm at the time of offspring-forming conjugations. The metabolic activity, growth and reproduction, of an organism are not unlimited, each species having its limit of vitality. As more water cannot be forced into a jug that is already filled, so it is impossible, under constant temperature conditions, to increase vitality in protoplasm that is already functioning to its full capacity. In *Uroleptus,* however, conjugations do not occur when the protoplasm is at its maximum of vitality and the difference in intensity of vitality between parent and offspring depends upon the age of the former at the time of conjugation. With offspring from young parents the parental vitality is relatively high and the difference in intensity for the first sixty days of life of the offspring between parent and offspring, is frequently so small as to fall within the limits of fluctuating variations or of experimental error. This was the case for example in Series 2, 4, 64, 71, 78, 79, 85, 96, 97, 102, 104, and 111 where the difference in intensity is less than two divisions in ten days. Reference to column 6 of the Table shows that all of these series came from young parents. Such slight differences afford little positive evidence of rejuvenescence and failure to take into account the age of parents explains a number of discordant results in the literature of this subject. With advancing age of the parent protoplasm the difference in intensity between parent and offspring becomes more pronounced. The young ex-conjugant returns to the full capacity of the species while the parent protoplasm shows the vitality

SYNOPTIC TABLE OF SERIES OF UROLEPTUS MOBILIS.

Offspring.				Parents.			Intensity.			
		Age.					First 60 days after offspring.			
Series, 1.	Relative vitality per cent, 2.	Divisions, 3.	Days of division, 4.	Series, 5.	Age, 6.	Divisions after offspring, 7.	Offspring, 8.	Parents, 9.	Difference, 10.	
1	90.9	313	267	...	...	...	17.3			
2	97.7	349	294	1	78	235	17.2	15.5	+ 1.7	
3	73.6	271	215	1	137	176	17.2	14.0	+ 3.2	
4	94.1	317	256	2	86	263	17.2	16.0	+ 1.2	
5	81.2	291	253	Cyst	...	...	17.5			
6	78.6	268	245	1	237	76	16.8	9.1	+ 7.7	
7	89.0	322	238	4	143	174	17.1	4.4	+12.7	
8	69.0	253	197	1	311	2	17.9	1.0	+16.9	
9	74.5	273	210	7	199	123	17.3	15.0	+ 2.3	
10*	68.5	250	196	Cyst	...	...	18.7			
11	71.3	257	188	8	188	65	18.4	8.9	+ 9.5	
12	79.5	268	197	10	108	...	18.2	14.8	+ 3.6	
13	95.0	327	287	9	116	157	15.6	12.0	+ 3.6	
14	5.4	23	35	7	316	6	3.4	0.6	+ 2.8	
15	48.1	180	185	8	245	8	13.9	0.8	+13.1	
16	76.3	285	236	9	208	65	12.3	6.9	+ 5.0	
17	85.9	301	255	9	208	65	12.7	6.9	+ 5.8	
18	88.1	317	265	13	120	207	15.5	12.4	+ 3.1	
19	110.4	597	598	11	225	32	10.4	1.0	+ 9.4	
20	71.8	281	286	13	135	192	11.8			
21	81.3	313	376	13	158	169	14.3	14.2	+ 0.1	
22	64.5	264	291	13	158	169	16.7	14.2	+ 2.5	
23	89.3	328	225	9	208	65	13.1	5.6	+ 7.5	
24	57.8	226	168	18	270	47	15.0	3.8	+11.2	
25	52.7	191	165	22	183	99	14.0	6.6	+ 7.4	
26	42.7	163	125	22	205	59	15.2	7.7	+ 7.5	
27	46.3	181	123	22	205	59	15.5	5.4	+10.1	
28	52.2	202	163	18	270	47	15.6	3.8	+11.8	
29	51.5	192	136	18	270	47	14.5	3.8	+10.7	
30	31.8	120	101	18	270	47	15.0	3.8	+11.2	
31	51.3	202	140	18	270	47	16.0	3.8	+12.2	
32	44.0	151	148	13	291	36	12.4	4.0	+ 8.4	
33	43.6	137	120	32	64	...	14.4			
34	.....	...	10	33	80					
35	94.6	370	311	23	208	120	19.7	15.2	+ 4.5	
36	78.2	302	318	23	248	80	21.3	8.5	+12.8	
37	42.6	177	103	34	147	...	19.6	6.4	+13.2	

38	55.1	192	266	35	92	278	20.6	18.6	+ 2.0
39	48.9	187	205	28	176	26	19.8	3.1	+16.7
40	87.0	302	270	35	112	258	20.0	18.9	+ 1.1
41	81.2	286	219	19	235	...	20.3	16.8	+ 3.5
42	50.0	187	134	37	70	107	18.5	16.4	+ 2.1
43	90.9	316	268	19	293	...	18.6	16.0	+ 2.6
44	39.5	147	199	19	332	...	12.2	12.1	+ 0.1
45	105.0	394	357	39	94	93	13.6	8.9	+ 4.7
46	90.9	320	330	40	90	212	15.2	12.4	+ 2.8
47	5.9	20	42	24	224	2	2.3	0.2	+ 2.1
48	79.5	274	265	41	93	193	13.6	13.6	0.0
49	65.0	238	205	40	140	162	13.8	10.7	+ 3.1
50	79.0	319	252	41	139	147	13.4	11.0	+ 2.4
51	70.4	267	184	48	51	223	13.5	11.4	+ 2.1
52	29.0	105	139	51	89	178	13.6	16.7	− 3.1
53	60.0	214	194	45	142	252	16.5	12.6	+ 3.9
54	50.0	210	211	52	42	63	15.3	8.8	+ 6.5
55	74.0	262	253	50	160	159	15.0	10.5	+ 4.5
56	61.3	193	222	53	121	93	13.4	11.8	+ 1.6
57	60.0	219	317	49	226	12	14.7	3.8	+10.9
58	44.5	167	148	53	157	57	14.3	7.0	+ 7.3
59*	82.7	250	295	57	54	165	14.9	6.9	+ 8.0
60	71.3	270	296	59	51	...	16.5	14.4	+ 2.1
61	97.3	319	344	59	104	...	15.9	11.2	+ 4.7
62	96.4	316	398	60	97	173	13.6	13.6	0.0
63	31.1	123	120	56	170	23	13.7	2.0	+11.7
64	91.4	346	344	61	74	245	13.6	12.2	+ 1.4
65	92.7	354	369	61	110	209	14.5	12.0	+ 2.5
66	69.1	233	237	59	181	...	13.4	5.2	+ 8.2
67*	36.3	134	106	63	Cyst	...	15.3		
68*	30.9	138	143	63	Cyst	...	13.5		
69	99.1	350	334	64	119	227	17.6	11.8	+ 5.8
70	95.0	350	355	66	141	92	15.0	10.2	+ 4.8
71	83.6	314	288	69	116	234	11.6	10.6	+ 1.0
72	81.8	312	289	64	219	127	10.8	7.2	+ 3.6
73	76.8	288	286	64	225	121	10.4	7.2	+ 3.2
74	.....	...	...	...	...	...	....	....	.....
75	.....	...	...	...	...	...	....	....	.....
76	.....	...	...	...	...	...	....	....	.....
77	89.1	334	298	70	96	254	13.1	10.8	+ 2.3
78	75.0	288	260	71	89	225	13.8	13.2	+ 0.6
79	65.9	291	218	72	115	197	10.6	10.4	+ 0.2
80	65.0	256	236	73	105	183	12.4	10.0	+ 2.4
81	51.8	206	209	74	132	...	11.2	10.6	+ 0.6
82	54.1	218	206	78	42	246	11.4	12.6	− 1.2
83	68.1	239	195	76	140	...	12.1	11.8	+ 0.3
84	73.6	261	194	78	86	202	14.2	12.2	+ 2.0
85	90.4	319	334	82	35	183	12.4	11.2	+ 1.2

Series discarded after fifty days; same as 71.
Failed to reorganize.
Discarded after fifty days; same as 77.

* = Cysts.

SYNOPTIC TABLE OF SERIES OF UROLEPTUS MOBILIS.—(*Continued.*)

Offspring.				Parents.			Intensity.			
		Age.					First 60 days after offspring.			
Series, 1.	Relative vitality per cent, 2.	Divisions, 3.	Days of division, 4.	Series, 5.	Age, 6.	Divisions after offspring, 7.	Offspring, 8.	Parents, 9.	Difference, 10.	
86	.....	...	...	...	...	...	....	....	.....	Series given away.
87*	.....	...	...	...	...	...	....	....	.....	Series lost by accident.
88	87.2	305	236	85	135	184	13.6	10.6	+ 3.6	
89	103.6	367	405	87	68	...	12.0	13.6	− 1.6	Double organism.
90	98.1	356	290	87	88	...	15.3	12.6	+ 2.7	
91	76.9	310	274	89	278	89	15.4	11.5	+ 3.9	
92	87.0	329	222	91	52	258	16.0	13.7	+ 2.3	
93	93.6	350	256	91	70	240	15.8	13.7	+ 2.1	
94	92.2	335	313	88	149	156	13.6	10.8	+ 2.8	
95	92.4	350	297	90	168	188	17.2	13.2	+ 4.0	
96	83.6	348	301	93	123	227	15.0	14.6	+ 0.4	
97	79.1	299	285	94	105	230	16.2	14.3	+ 1.9	
98	86.0	308	245	97	101	198	16.0	13.8	+ 2.6	
99	79.5	299	219	95	171	137	18.7	13.8	+ 4.9	
100	.....	...	...	...	...	...	....	....	.....	Died in twenty days; reversion from series 89.
101	66.0	299	162	98	92	216	13.6	10.6	+ 3.6	
102	92.7	348	259	99	93	206	15.0	13.2	+ 1.8	
103	67.2	248	267	99	205	94	15.7	11.7	+ 4.0	
104	70.3	266	236	102	111	237	19.0	19.0	0.0	
105	76.6	289	259	98	186	122	18.7	12.5	+ 6.2	
106	44.3	181	193	105	116	173	12.9	11.6	+ 1.3	
107	15.4	56	55	102	247	101	7.7	8.1	− 0.4	
108	.....	...	...	...	...	...	....	....	.....	Series failed to reorganize.
109	49.3	201	162	105	185	104	10.9	7.5	+ 3.4	
110*	63.5	236	267	105	...	...	11.3	6.4	+ 4.9	
111	74.5	313	256	110	72	164	10.8	9.6	+ 1.2	
112	77.6	316	256	110	72	164	9.1	9.6	− 0.5	
113	6.2	35	34	110	125	111	4.4	7.6	− 3.2	
114*	53.6	194	224	111	...	...	8.3	9.4	− 1.1	
115	81.3	284	221	109	174	27	10.5	2.8	+ 7.7	
116	87.1	311	278	111	125	188	12.8	12.2	+ 0.6	
117	86.3	312	292	115	48	236	13.4	10.4	+ 3.0	
118	88.1	332	287	112	148	168	19.3	15.2	+ 4.1	
119	50.8	188	134	114	75	119	17.6	13.1	+ 4.5	
120	.....	...	...	117	130	182	15.2	8.6	+ 6.6	Still living.

characteristic of its age. The difference between them is now beyond the range of fluctuating variations or of experimental error and furnishes unmistakable evidence of rejuvenescence. Series 7, 11, 24, 27, 28, 29, 30, 31, 36, 57, and 63 which exceed their parents, in rate of division by from 8 to 10 divisions per ten days illustrate this point, and reference to column 6 shows that these series came from parents well along in age. With extremely old parents finally the difference in intensity between parents and offspring reaches its maximum and if parents have less than 35 divisions subsequent to their age at the time of conjugation (column 7), the offspring have an intensity of from 11 to 16 divisions per ten days more than the parent protoplasm (Series 8, 15, 39, 63).

3. **Relative Vitality of Different Series and Effect of Parents' Age on Vitality of Offspring.**— Do ex-conjugants from old parents have as much vitality as do ex-conjugants from young parents? That is, is the organization of offspring affected by the depleted vitality of the parent? Except in extreme cases these questions cannot be answered by comparison of the intensities of vitality of the two series. For example a series living two hundred days and dividing 300 times would have an average intensity of vitality indicated by 15.0 divisions in ten days; another series living only fifty days and dividing only 75 times likewise has an intensity of 15.0 divisions per ten days. It would be far from exact to say that the two series have the same vitality; here the time factor or endurance is not taken into account. Hence to compare vitalities of two different series both intensity and endurance must be represented. The method adopted (Calkins 1920) rests on the principle of reference to a common, ideal life cycle represented by a numerical constant. The number of generations by division and the days of life of a series have a definite relation expressed by a percentage of such an ideal constant. Such percentages indicate the relative vitality of the different series and are listed in column 2 of the Table.

With these percentages expressing relative vitality it is possible to compare different series in respect to the effect of age of parents on the vitality of offspring. There is unmistakable evidence contained in the Table that offspring from old parents in the great majority of cases have a much lower relative vitality than do the parental series, or series from young parents. This is best illustrated by instances where two or more offspring series are taken off at different periods in the life history of the same parent. Such a sequence is illustrated by Series 2, 3, 6 and 8, all of which came from Series 1, and with a difference of 28.7 per cent in relative vitality between the first (Series 2) and the last (Series 8) offspring. Another striking illustration is shown by Series 7 and its two offspring Series 9 and 14; Series 9 came from Series 7 when the latter had lived more than half of its life and its relative vitality was about 15 per cent

lower than its parent. Series 14 came from the same parent when the latter had only 6 more divisions in its life history and the effect of its old age is shown by the relative vitality of 5.4 per cent of its offspring, Series 14. It is quite evident that the protoplasmic organization of the parent is not the same at the beginning and at the end of its life and that the effect of the change is indicated by the organization and activities of its offspring. Some interesting and perhaps significant surprises have turned up however from such old age conjugations and it is possible that mutations may arise at such times. Thus Series 19 came from parents that were 225 generations old and with only 32 more generations to live. The expectation would be a low relative vitality for this old age offspring, but on the contrary it had a relative vitality of 110.4 per cent, the highest on record (see p. 580 for further consideration of this case.)

In our experience it has been impossible to restore an extremely weak series to a vigorous condition by conjugation, all such attempts result in still weaker series. It is possible, however, to restore comparatively weak series to full strength, a result which Woodruff and Spencer also obtained with *Spathidium spathula*. This is well shown by Series 60 and 62, in which the relative vitality is raised from 70.3 to 96.4, or by Series 66 and 70, in which it is raised from 69.1 to 95.0, etc.

4. **Rejuvenescence After Parthenogenesis (Endomixis).**—Woodruff's long culture of *Paramecium aurelia* furnishes an excellent illustration of continued vitality through reorganization by parthenogenesis. The fluctuations or waves in his graph (Woodruff 1921) indicate a series of depressions followed by increased vitality; reorganization occurs during the periods of depression. Different culture media have no effect in changing the frequency of endomixis in time but may cause an increase or decrease in the number of interendomictic generations by divisions (Woodruff, 1917). According to Jollos (1916) external factors may call out parthenogenesis in *Paramecium* at any stage in the life history, and according to Young (1917) sudden sharp changes of medium may bring on endomixis prematurely, but the sequence always lapses to the regular routine and usually by the next period. If endomixis does not occur the race invariably dies. "This indicates strongly, if it does not prove that a periodic occurrence of the definitive endomictic phenomena is a *sine qua non* for the continued life of the race" (Woodruff, 1917, p. 462).

With *Uroleptus mobilis* the evidence for rejuvenescence through parthenogenesis is of the same kind as that from conjugations. Reorganization without fertilization takes place during encystment and the cysts are formed early in the life history of a series (see p. 490). On emerging from its cyst the organism is treated as though it were an ex-conjugant and the first five individuals are maintained as five pure lines of the series. Such series are indicated

in the Table, p. 560 by an asterisk. The vitality of the first sixty days of a cyst series is compared with that of the parent series for the sixty days following encystment and the results are practically the same as with ex-conjugants. In some cases the cysts are kept dried for a period of weeks or months but this has no effect upon the vitality of the organism when it emerges. In all cases the evidence of rejuvenescence is the same as for ex-conjugants from young series.

The general results of these experiments with *Uroleptus mobilis* leave little ground for reasonable doubt of the rejuvenating effect of conjugation. The view of Woodruff and Spencer (1924) that loss of vitality and death here are due to conditions of the milieu seems rather far-fetched when we consider that series after series with the similar sequence of renewed, waning, and exhausted

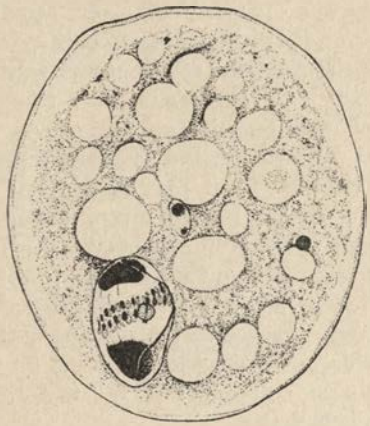

FIG. 233.—*Karyamœba falcata.* (After Kofoid and Swezy.)

vitality pass by in apparently endless succession, and all in the same milieu so far as it is possible to make it the same, from the beginning of the experiments eight years ago to the present. It is quite a different question whether or not conditions of the medium can be so altered as to bring about the same results as conjugation. The explanation must be looked for in the protoplasmic happenings at the period of conjugation or of endomixis (see Chapter XI). In both cases these result in a rearrangement of the chromatin and cytoplasm which according to Erdmann (1921) gives rise to new sets of autocatalyzers and new cytoplasmic matrices for their activation.

Nothing is known about the effect of encystment on vitality in the Sarcodina or Mastigophora. There is no *a priori* reason to doubt that as in ciliates reorganization is accomplished during

such stages in the life history. The described cases of autogamy during encystment of *Endamœba coli* and *Endamœba muris* (see Chapter XI), although the facts are doubted by many, is evidence in this direction. So also are the peculiar fertilization phenomena in *Amœba diploidea* (Fig. 230), or the presence of mitotic figures in encysted Rhizopoda, for example *Karyamœba falcata* (Kofoid and Swezy, 1924; Fig. 233).

The general and philosophical aspects of the phenomena described above, particularly those pertaining to the so-called physical immortality of the ciliates, are important or not according to the individual point of view. To my mind the phenomena in these forms lead to the conclusion that Protozoa and Metazoa are fundamentally alike in respect to protoplasmic continuity and protoplasmic death, the difference between them is bound up with our definitions of the "individual." So far as immortality of Protozoa is concerned, Hertwig's (1914) conclusions appear to sum up the situation: "However these investigations may turn out, one may say this now, that the doctrine of the immortality of the Protozoa in the form established by Weismann at a time when we did not know anything of the fertilization processes of the Protozoa, cannot be retained. The beautiful investigations of Erdmann and Woodruff do not detract from my conception based on former work and repeated here, but furnish a new affirmation that death in imany-celled animals is the result of peculiarities which are present in everything that is alive, and that the life process contains within itself the germ of death and that the harm connected with it (death) may be postponed in Protozoa by reorganization processes. In many-celled animals however, these cannot be applied, the more the life of the single cell depends on the total organization." (Hertwig, 1914, p. 580.)

II. HEREDITY AND VARIATIONS IN PROTOZOA.

Owing to the relative simplicity of the organisms with which we are dealing there are few structural characteristics that can be used in a study of variations. Variations in size are often noted but these in themselves do not furnish reliable data, a *Dileptus anser* for example may be 250 microns in length or only 25 microns (Fig. 6, p. 28) according to the food it gets. Similar differences due to temporary conditions are evident in all organisms that are studied for a sufficient length of time. In a mixed population, however, size differences may indicate fixed variations as was clearly shown by Jennings (1909) for *Paramecium* (Fig. 234).

It is difficult to distinguish between fluctuating or cyclical variations and germinal variations and the distinction cannot be realized where the germinal history is unknown. The difficulty

is increased by the fact that comparatively few life histories of Protozoa are known. Many variations that have been recorded may be cyclical in nature and repeated in all life histories of individuals of the species. These correspond to differentiations in ontogeny of Metazoa and have been more fully discussed in Chapter X. The fact that such variations breed true by cell division is to be expected for the organism could not do otherwise. The test comes with amphimixis or parthenogenesis.

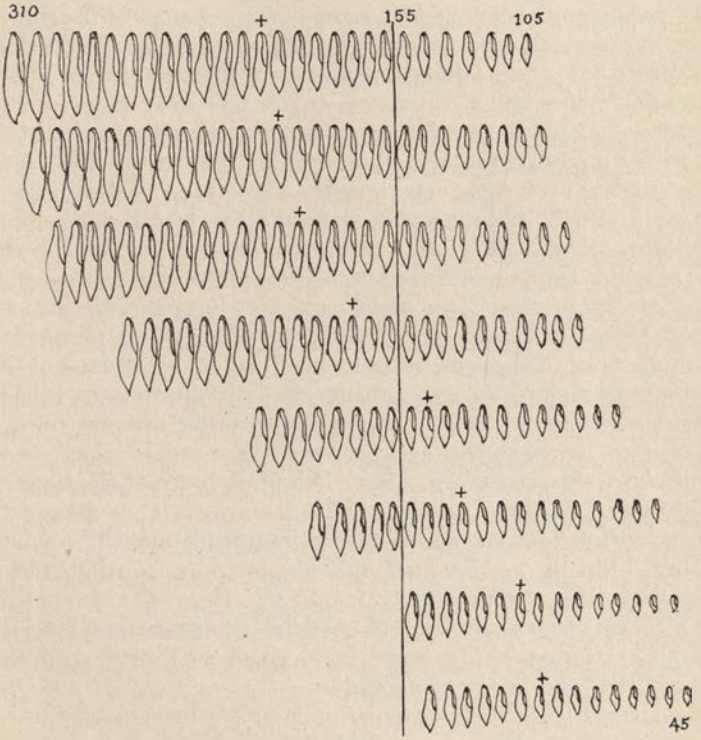

FIG. 234.—Size variations in eight families of *Paramecium*. (After Jennings.)

A. Uniparental Inheritance.—It is quite possible that changes in the genotype or organization of Protozoa may occur and remain permanently, and such changes may be due to environmental or to internal causes. Changes due to environmental causes, to be permanent, would have to so affect the germinal make-up that reversions would not occur. Thus individuals formed by reversions from the double *Uroleptus* described in Chapter X (p. 465) never regenerated the double organism but lived as single individuals of *Uroleptus mobilis* (Series 91 of Table, p. 560). Here the organi-

zation was unchanged although the new double type of organism lived for four hundred and five days and divided 367 times.

Variations due to environmental changes should be retained as long as such changes are maintained. Thus Zuelzer obtained a very different type of organism by transferring *Amœba verrucosa* from fresh to salt water. The variation lasted as long as the organisms were kept in salt water but reverted to the original form on transference to fresh water again. Jennings (1921) cites a number of cases of bacteria in which the organization appeared to be permanently changed by a temporary change of drastic character in the medium. Similar results have been obtained with Protozoa where adaptations or responses of the organism to solutions of gradually increasing concentrations or to slowly increasing temperature changes have apparently become permanent, or at least endure for many generations by division. Among the first, and the more extensive of such experiments, were those of Dallinger and Drysdale (1873) in connection with the life histories of different flagellates. Dallinger (1907) in particular, working with remarkable patience and perseverence for seven years was able to accustom three species of flagellates which are described as *Tetramitus rostratus*, *Monas dallingeri*, and *Dallingeria drysdali* to temperatures which are fatal to these organisms under normal conditions of 60° F. At the beginning of the experiment all individuals were killed by a sudden change to 78° F., but by accustoming them to slowly increasing temperatures acting for long periods they became adapted to this condition. Such adapted individuals were then subjected to further increases in temperature, the change from one degree of heat to another often requiring months of patient waiting. Finally he obtained individuals which continued to live vigorously in a temperature of 158° F. Here was a change in organization or an adaptation to changed conditions which persisted as long as the conditions were maintained and until an accident brought the experiment to an end.

Similar but less extensive experiments have been carried on with other Protozoa. Within the last decade Middleton (1918) and Jollos (1913, 1923) have tested the effect of increased temperatures on ciliates. Middleton (1918) separated progeny of an individual of *Stylonychia pustulata* into two groups one of which was kept for some thirty days at a relatively high temperature (about 30° C.) the other at a low temperature (10° C.). The set at 30° C. divided more rapidly than those at 10° C. They were then transferred to a common intermediate temperature in which the previously warmed individuals continued to divide more actively than the cooled set. Evidence of the same type is furnished by the interesting experiments of Hartmann (1924) on *Eudorina elegans* and *Gonium pectorale*. By use of potassium nitrate and ammonium chloride

in the nutrient medium and under conditions of sunlight or of artificial light he was able to cause a change of the thirty-two-cell colony *Eudorina elegans* into the flat, colonial sixteen-cell type of *Gonium pectorale,* and *Gonium pectorale* into *Eudorina elegans.* With return to the normal medium all such changed organisms reverted to their respective genotypes.

Experiments of this type and others to be described below show that changes in organization can undoubtedly be produced in Protozoa. If such changes are permanent they may be interpreted as mutations; if not permanent they have little more value than the fluctuating variations which accompany changes of metabolism. The great majority of changes which have been described are centainly not mutations but illustrate the flexibility of protozoan organizations and broaden the limits within which fluctuating variations are known to occur. Such variations ultimately revert to type and although they may last for many generations by division, they have no permanent effect upon the organization. Jollos (1913) terms them "enduring modifications" (Dauermodificationen). Other frequently-cited illustrations of this type of variations have to do with the effects of minute doses of poison on the organization. Some races of *Trypanosoma* for example, may become adapted and immune to weak doses of arsenic—the so-called poison-fast, arsenic-fast, atoxyl-fast races first described by Ehrlich. Bignami (1910) thus interprets malaria relapses as due to quinine-fast organisms. Such modified types retain their immunity for long periods and through many successive generations of transplants but they apparently belong to this type of enduring modifications. Gonder (1912) has shown that poison-fast races of *Trypanosoma lewisi,* lose their acquired immunity by passing through the rat flea. Also races of *Trypanosoma* without parabasal bodies (Blepharoplastlose) first obtained by Werbitzski (1910) by injecting pyronin into the host's blood, would live for many generations of transplants without this kinetic element, but the parabasal body ultimately reappears. Here too in all probability, belong the so-called mutations in *Ceratium vultur* described by Kofoid (1908), and those in Radiolaria described by Haecker (1909) the observations in both cases being somewhat casual and not followed up experimentally so that the matter of permanency is in doubt.

The extensive experiments on *Paramecium* made by Jollos (1913, 1923), offer many illustrations of change in organization and subsequent return to normal, sometimes after many vegetative divisions, sometimes after endomixis, and again only after conjugation. The effect of arsenic acid calcium compounds, and extreme temperatures, were lasting through one or more periods of endomixis and conjugation, but such effects were ultimately lost. A significant fact however is the difference in effect produced by treatment with

arsenic or heat at different periods. If treated during vegetative life the results were as described above, *i. e.*, temporary changes or enduring modifications. If treated during the later phases of conjugation, that is, during the period of reorganization of the ex-conjugant (Jollos calls it the "sensitive" period) then the effects were found to be permanent in a very small percentage of cases. Such changes are evidence of a change in the organization itself, or in the genotype, and were found to be lasting for generations by conjugation. Jollos is apparently right in speaking of such cases as mutations.

In this connection also we should include the numerous attempts to perpetuate abnormalities in Protozoa. Popoff (1909) by centrifuging *Stentor* when about to divide produced individuals in which the original beaded nucleus was unequally distributed, one individual receiving 16 beads the other only 3. Both individuals reorganized perfectly after fusion but the one with 3 beads was about one-quarter the size of the individual with 16 beads. The two types were persistent and divided normally for a short time, the progeny of the smaller form regenerating the normal number of beads. The cultures were then lost so that the further history is unknown. In another case a dividing *Stentor* was suddenly cooled so that the division processes ceased. The individual was then placed under conditions of normal temperature, conditions where it reorganized into a single but very large individual. From it a race of giant *Stentors* was obtained by reproduction, the individuals breeding true for a period of about six weeks. An analogous experiment by Chatton (1921) was made on the ciliate *Glaucoma scintillans*, by treating individuals in the early phase of division with a dilute solution of sodium bromide (16 to 1000) for ten minutes. The division processes were hastened by the change in osmosis and when nearly divided the individuals were restored to their normal medium where the division planes were lost and the two nearly divided halves were again resolved into one. In this manner Chatton obtained individuals with two mouths, several micronuclei and only one macronucleus each. On reproduction some of the offspring were similarly distomous, while some, as with the *Uroleptus mobilis* double individual, reverted to the single type. The double individuals were maintained in culture for a period of five months (sic) when they were abandoned, Chatton believing that they might be continued indefinitely by division. Analogous double individuals were obtained by Dawson (1920) by the fusion back to back of amicronucleate individuals of *Oxytricha hymenostoma*. The double individuals reproduced double individuals for 102 generations by division. Dawson's monsters ultimately died. The permanence of Chatton's *Glaucoma scintillans* may well be questioned and it is unfortunate that he discarded the race after only five months of

culture. The double *Uroleptus* at the age of five months was more vigorous than at the outset, but like all other series of *Uroleptus* it ultimately died. It lived and reproduced however for more than fourteen months (see p. 465).

Similarly with mutilations. The mutilated portions are passively handed down to progeny by division, but the organization is not affected and in the course of a few divisions the normal type is regenerated. This was demonstrated by Jennings (1908) and confirmed by Calkins and by Peebles (1911, 1912) in cutting off the anterior or posterior end of *Paramecium* leaving a truncated individual which did not regenerate but divided to form a perfect individual from the posterior end and a truncated individual from the anterior end (Fig. 103, p. 219); after a few divisions both anterior and posterior individuals were perfectly normal. Abnormal projections such as spines or clefts in the cortex, etc., are likewise passively transmitted to descendants by division for a limited time, but no permanent change in organization is brought about.

In general the upshot of all experiments with poisons, heat, abnormalities, etc., is failure to modify the organization of Protozoa in any permanent manner. The experiment of Jollos of treating *Paramecium* at the time of reorganization is, however, a possible exception.

Modifications of the organization which arise from within the organism itself, on the other hand, may be permanent. Such modifications are possible through the sifting out of germinal characteristics in the course of continued metabolic activity and division. Some are manifested by morphological characters which afford a basis for selection on the part of the investigator. Experiments to this end have been carried out mainly by Jennings and his associates. The underlying principle in such selection work is that a single individual from a "wild" population is the result of a great number of hereditary characteristics stored up in the past through amphimixis and united now in the organization of the single individual. Such an individual, if cultivated under uniform conditions, gives rise to progeny showing diversities in structure or function which are probably ancestral characters. The extreme individuals showing such diversity are selected and bred independently.

Jennings has clearly shown that such differences are characteristic of all the pure lines he has studied and his findings have been confirmed by Root (1918) for *Centropyxis aculeata;* by Hegner (1919) for *Arcella dentata;* and by Reynolds (1923) for *Arcella polypora.* While the fundamental character (genotype) of a race is maintained there are minor differences in organization which may or may not be manifested by structural peculiarities. This is strikingly shown in Jennings studies on *Difflugia corona* (1916) a favorable form since the characteristics of the shell can be measured or counted and the

structure does not change after it is once formed. In such a study Jennings says the method of evolution by slow and gradual change rather than by sudden jumps or mutations becomes visible. "We begin to exercise selection within the single family. On the one hand we select all the long-spined individuals and place them together; on the other hand we select all the short-spined ones and place them together. In the long-spined group we continue to save for generation after generation only the individuals that are long-spined; in the short-spined group only the offspring with short spines. In the same way we select other sets for numerous spines and for few spines; for large shells and for small shells; for many teeth and for fewer teeth.

"And now as we keep this up for generation after generation we find that the correspondence between parent and progeny becomes more and more marked. We find that our single family is breaking up into many different groups which differ from one another hereditarily. We get finally what appears to be two diverse races—one with long spines, the other with short spines—the difference continuing for generation after generation. A third set has constantly large shells, while others consistently produce small shells. We also get stocks hereditarily different for numbers of spines; and for numbers of teeth. Our single stock derived by fission from a single parent, has gradually diversified itself into many stocks that are hereditarily different. If this is what we mean by evolution, we have seen evolution occur" (Jennings 1921, pp. 75–78).

In a similar manner Root (1918) and Hegner (1918) studied uniparental inheritance in *Centropyxis aculeata* and in *Arcella dentata* and obtained results of the same nature. External agents (lack of food, salts, temperature, etc.) may bring about similar variations in size of shell, numbers of spines, etc., which persist as long as the conditions are maintained (Hegner, 1919). From this it appears that external conditions may inhibit the expression of germinal factors, but not permanently.

The interpretation as given by Jennings of these clear-cut results appears to be fundamentally sound and its significance is not lessened by the chromidia problems which are associated with all of these testate rhizopoda. If as generally believed, the chromidia give rise to germ nuclei there is some chance of this hereditarily important chromatin being unequally distributed at cell division, for the mass of chromidia is not halved with the same precision as is the chromatin of the nucleus or nuclei. Whether or not chromidia are responsible the interesting fact remains that demonstrable variations in organization occur with continued reproduction. It remains to be determined, however, whether the variations will still breed true after endogamous fertilization and reorganization, or will revert to the form of the original wild individual; then only

will the matter of permanency of the changed organization be settled. Jollos (1924) exercising selection in *Arcella vulgaris*, *Arcella discoides*, and *Arcella polypora* obtained abnormalities in parents and offspring which he interpreted as due to environmental conditions especially to the accumulations of metabolic waste. With cultivation under better conditions of the medium such abnormalities gradually disappeared with reversion to the normal.

Further evidence of the sorting out of mixed characteristics was given by Calkins and Gregory (1913). The first 4 of the individuals formed by an ex-conjugant of *Paramecium caudatum* were individually isolated and the history of their progeny was followed out in 32 pure lines, 8 from each of the original 4 individuals. The history of these 4 strains in one experiment is condensed in Fig. 235. Pure lines that died are indicated by X and the 4 sets of 8 lines each came from the 4 individuals A, B, C, and D. Physiological differences in the progeny of these 4 are indicated by the division-rates and by the ability to conjugate, the progeny of A for example giving epidemics of conjugation at each test while similar tests gave no conjugations in the progeny of B, C, and D until nine months of culture and then in very small numbers. Similar variations in size were characteristic of the different quadrants. It is possible that such results are due to the segregation of germinal materials during three metagamic divisions of the amphinucleus, each of the original four cells receiving a different combination of macro- and micronuclei.

Selection on the basis of physiological activities alone is not always satisfactory in results. Middleton (1915) for example, continually selecting progeny of *Stylonychia* for rapidity and slowness of division obtained two sets with what he regarded as an inherited difference in rates of division and the permanence was tested by conjugation—the ex-conjugants being followed for fifteen days. "Thus," he concludes, "it is clear that the heritable difference in fission-rate brought about by selection during vegetative reproduction is not lost when the animals conjugate, but persists through that ordeal" (1915, p. 497). Not only was the period of observation of ex-conjugants too short for a conclusion of such importance but the actual results justify an opposite conclusion. From his table we learn that 60 ex-conjugants from "fast" lines divided 1297 times in fifteen days, while 60 ex-conjugants from "slow" lines divided 1310 times during the same period. That is, his carefully selected slow lines after conjugation actually divided more times than did the selected fast lines. His remarkable conclusion was apparently based on the fact that on 3 of the 5 three-day periods his fast lines divided a little more rapidly than did the slow lines but not fast enough to overcome the lead obtained by the slow lines during the first two periods.

In general all results that are based upon physiological differences must be cautiously interpreted. Thus with *Uroleptus mobilis* individuals from the progeny of single ex-conjugants may be selected at appropriate periods to show marked differences in division-rates. One such individual may reproduce at the rate of

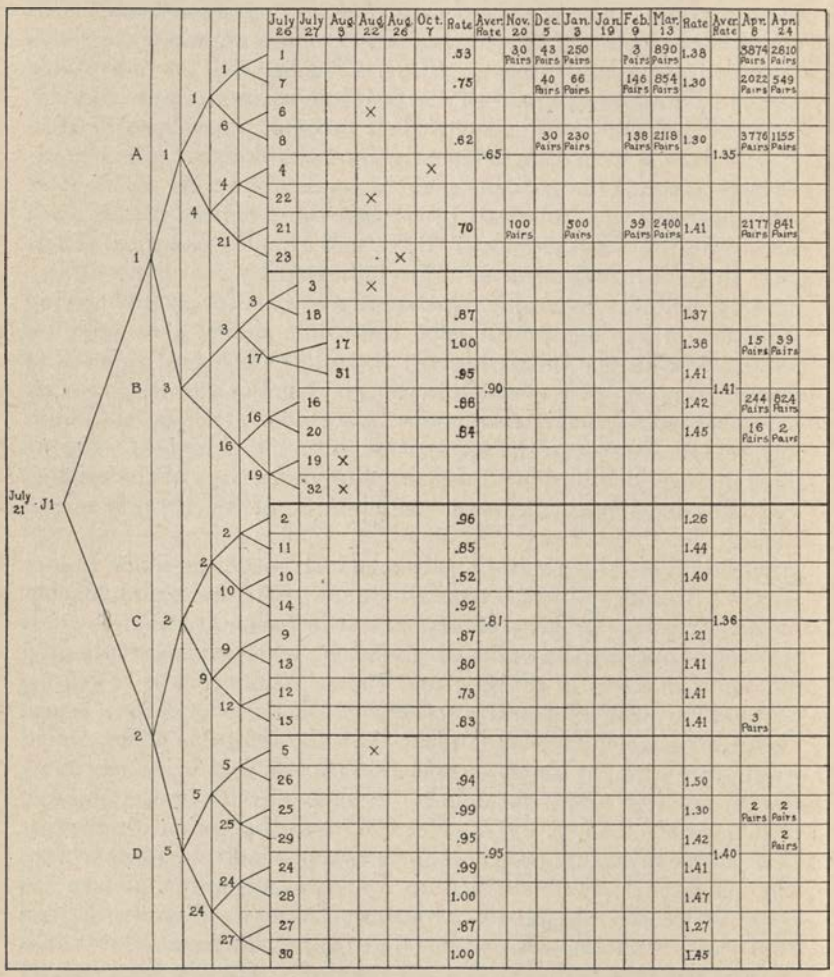

FIG. 235.—Variations in the progeny of a single ex-conjugant of *Paramecium caudatum*. (After Calkins and Gregory.)

17 divisions in ten days; another individual from the same line will reproduce at the rate of 8 divisions in ten days, and a third may divide at the rate of only 2 divisions. One might erroneously argue that these individuals represent the sifting out of an heredi-

tary complex and the argument would apparently be supported by results of conjugation between individuals of each set. In the first set the high division-rate would appear to be inherited; in the third set the low division-rate in most cases would appear to be inherited but such series invariably die. The real test is shown by conjugation in the second set which results in optimum division-rates. In such sets of progeny, as shown above, the differences in vitality of the offspring through conjugation are due to differences in vitality of the parent. With low vitality of offspring from old parents it might be argued that here is an example of the inheritance of an acquired characteristic whereas it is merely a matter of general vitality.

B. Biparental Inheritance.—Through amphimixis there is a possibility of introducing changes in the organization of a species from within. The new amphinucleus is a new creation and its interaction with the cytoplasm must differ from previous interactions. The cytoplasm is also different in cases of merogamy and in cases of conjugation. In merogamy there is a fusion of cell bodies as well as of nuclei; in conjugation the old macronucleus a product of the old amphinucleus, is distributed throughout the cytoplasm and absorbed. As a result of the interactions of new nucleus and new cytoplasm, new structures and new activities or changed activities may ensue.

While *a priori* such origination of variations in Protozoa is a logical consequence, as a matter of fact it has been rarely observed in Protozoa. Here genotypes as well as fixed and congenital variations usually vary little from the fluctuating variations of a species. The remarkable fixity of the genotype is indicated by the world-wide distribution of the common species, and is clearly demonstrated by long-continued cultures of any given species. Vitality also is remarkably constant as illustrated by Woodruff's long culture of *Paramecium aurelia*, by Hartmann's culture of *Eudorina elegans*, or by cultures of *Uroleptus mobilis* in which the average relative vitality of the first 12 series representing the F to F_4 generations by conjugations was 83 per cent and the relative vitality of a recent set of series representing the F_{18} to the F_{22} generation, was 85.6 per cent. Here although there was an interval of six years between the two sets compared, the vitality remains practically the same.

Despite this constancy there is some unmistakable evidence of variations in the Protozoa. There is also, considerable evidence that has been misinterpretated as mutations. Among the latter, abnormalities in reorganization may be responsible for apparent mutations. Thus a bi-micronucleated, short, race of *Paramecium caudatum* was obtained as a result of conjugation of two normal individuals (Calkins, 1906). Its two micronuclei, shortened body and rounded posterior end were characteristic of *Paramecium aurelia* and the latter was erroneously interpreted as a mutation of *Paramecium caudatum*. The *aurelia* characters persisted for 45

generations by division when they were lost and reversion to the *caudatum* type occurred, presumably during a period of endomixis. In like manner we may account for the amicronucleate races of many ciliates (Hance, Moody, Dawson, Woodruff, etc.), the amicro-

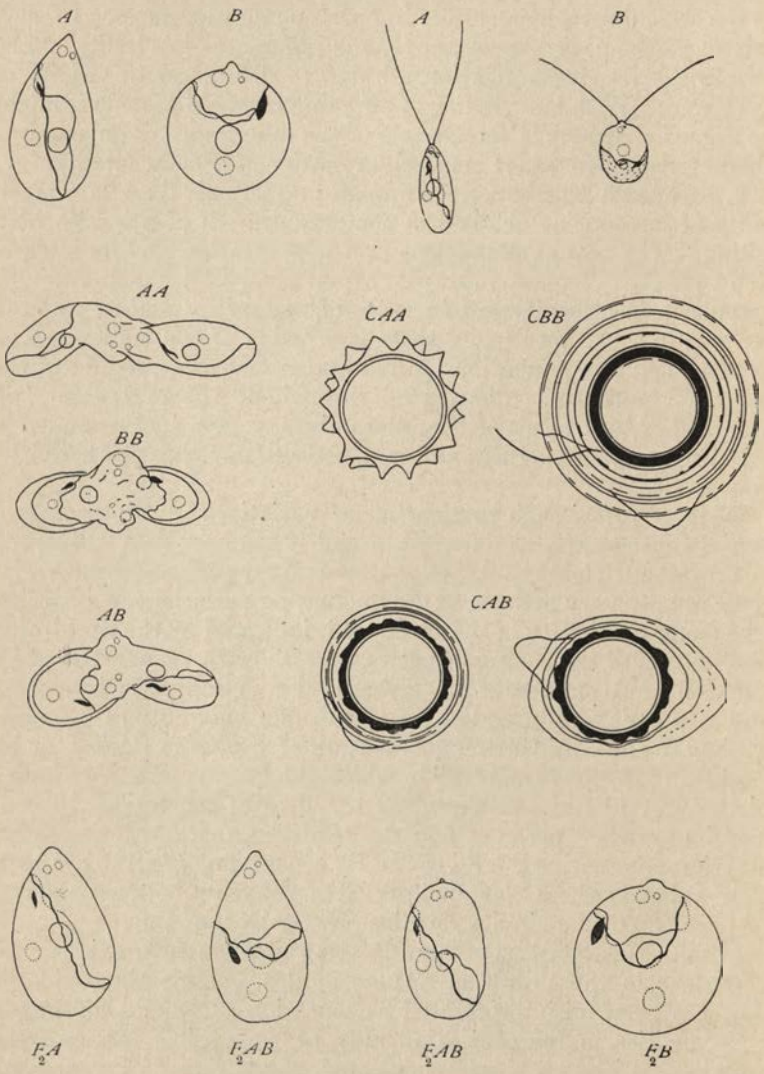

FIG. 236.—*Chlamydomonas* and Mendelism. *A*, one species of *Chlamydomonas; B*, a second species; *A'* gamete of *A; B'* gamete of *B; AA*, union of gametes of type *A; BB*, union of gametes of type *B; AB*, union of gamete of *A* with gamete of *B; CAA*, cyst formed by union of *A* gametes; *CBB*, cyst formed by union of *B* gametes; *CAB*, cysts formed by union of gametes from *A* and *B; F_2A*, one of the four products of the cyst *CAB*, and like *A; F_2B*, one, like *B; F_2AB*, two individuals of the four with combination of characters of *A* and *B*. (After Pascher.)

nucleate condition persisting for many generations but ultimately ending in death since failure to conjugate is characteristic of such races. These are evidently not cases of mutation but temporary abnormalities resulting from imperfect reorganization.

Variations following from Mendelian segregation have been repeatedly described among higher animals and plants; with Protozoa they are limited to one single case, described by Pascher (1916, 1918). Two different but not fully identified species of the phytoflagellate *Chlamydomonas* were cultivated in rich cultures. One of these (*A*) was characterized by a pyriform body, lateral, single chromatophore with lateral pyrenoid, two rather long, equal flagella, and a narrow dash-like stigma (Fig. 236). The other species (*B*) was more spherical and was characterized by a basal, single, chromatophore with basal pyrenoid; rather short flagella and a swollen stigma (Fig. 236, *B*). Both types reproduce by longitudinal division, and under appropriate conditions both types form isogametes. The differences in the two sets of gametes are of the same type as the differences between *A* and *B* (Fig. *A'*, *B'*). Fertilization is total (merogamic) and there is but slight difference manifested by the two types. Gametes of *A* shed their thick membranes on fusion, the old membranes remaining attached to the zygote as appendages (Öhrchen); gametes of *B* have a delicate membrane which is retained on fusion. The zygotes of the two species are also different; that of *A* is covered by a membrane ornamented by many conical warts (*CAA*, *CBB*); that of *B* is covered by several layers of smooth membranes. On germination of the zygotes 4 individual swarmers with the characteristics of their species emerge from both types of zygotes. Reduction in number of chromosomes evidently occurs in the zygote so that the ordinary vegetative individuals are haploid, the zygote alone diploid, and the 4 resulting swarmers are likewise haploid (zygotic reduction).

Pascher succeeded in crossing gametes of *A* and *B*, the heterozygotes having characters intermediate between those of the zygotes of *A* and *B* (Fig. *C,A,B; C,B,B*). On germination these heterozygotes gave rise to 4 different kinds of swarmers; 1 was like *A*, 1 like *B* and 2 were mixed as indicated in Fig. 236, and shown in the following table from Pascher (1918, p. 166).

	Form.	Membrane.	Chromatophore.	Stigma.
Chlamydomonas A	Spherical or pyriform.	Delicate; no papilla.	Lateral.	Narrow.
Chlamydomonas B	Spherical.	Thick: with papilla.	Basal.	Broad.
Swarmers from heterozygotes.	1. Pyriform.	Delicate; no papilla.	Lateral.	Narrow.
	2. Pyriform	Delicate; no papilla.	Basal.	Broad.
	3. Globular.	Thick; with papilla.	Lateral.	Narrow.
	4. Globular.	Thick; with papilla.	Basal.	Broad.

We have here a clear case of Mendelian segregation and a parceling-out of the germinal make-up in a definite Mendelian ratio which Pascher confirmed by direct observations in 5 different cases and by mass cultures in 8 others. The parental characters are sifted out and recombined to give organisms of a type different from either parent. In a single operation, and evidently through meiosis and fertilization, results of a similar nature to those obtained by Jennings after prolonged selection, were obtained.

Reference has already been made to the complete and elaborate studies of Jennings (1913) on inheritance as it applies to rejuvenescence (supra p. 556) and we refer here only to his results in regard to bi-parental inheritance (see also Jennings and Lashley 1913). No exception can be taken to the general conclusion that "Conjugation results in bi-parental inheritance" (Jennings and Lashley, p. 451). There is reason, however, for not accepting the experimental evidence that is adduced in support and proof of the conclusion. By experiment, observations, and use of biometric methods, Jennings studied elaborate data of conjugants, "split" conjugants, ex-conjugants and non-conjugants of *Paramecium caudatum* and *Paramecium aurelia* in respect to size, division-rate, abnormalities and mortality. He found that mortality is much higher in ex-conjugants than in non-conjugants or in split-conjugants; that the division-rate of ex-conjugants is uniformly less than that of non-conjugants or split conjugants; that abnormalities are more frequent among ex-conjugants than among non-conjugants or split-conjugants and that variations in respect to these "heritable" characters are greatest among the ex-conjugants. As to size there is no evidence at all: conjugationg individuals were shown by biometric methods to be smaller than non-conjugants of the same race (this is likewise true of most ciliates) but their progeny were not smaller. The best evidence given for his conclusions lay in matters of mortality, division-rates, abnormalities and variations of ex-conjugants. Dobell (1914) has pointed out some of the logical difficulties in connection with these studies but a more fundamental objection lies on the technical side. Quoting from Dobell: "But as Jennings himself points out 'conjugation increases the variation mainly toward the lower extremity of the range'—that is, the effect of conjugation is to retard the rate of fission. Is not this merely another aspect of the same condition which is otherwise manifested as 'high mortality' and 'loss of vigor' after conjugation? Jennings' Experiment 6 seems to me the key to the matter. 'This experiment as a whole shows the fact that after conjugation the organisms are in a condition such that many may die, while those that have not conjugated live; and the further fact that the rate of reproduction is made slower by conjugation, remaining in this condition for about two months' after which it has

'regained about the usual rate.' If the result of this experiment may be regarded as typical, then it indicates that the lowering in fission following conjugation is transient, recovery occurring sooner or later. It is demonstrated that after conjugation the organism and its progeny are weaker, or less resistant to external conditions (shown by higher mortality, lagging fission-rate, unstable size, abnormalities, etc.), for a certain time; and that complete recovery to the normal state preceding conjugation occurs subsequently; but I find no proof that from a race with a given fission-rate, another race with a permanently different fission-rate has arisen as a result of conjugation" (Dobell, 1914, p. 173).

The source of the difficulty with Jennings' conclusions lies in the cause of the transient lowering of the division-rate, of the increased mortality and of the increase in the number of abnormalities. Jennings finds the cause in bi-parental inheritance and in "incompatible combinations" at amphimixis, but a more probable cause, as Dobell intimates, lies in the conditions of the experiments and, as there is reason to believe, in the use of a culture medium which is unsuitable for *Paramecium* during the critical reorganization period after conjugation (see Calkins, 1923). It is unnecessary to repeat here all of the evidence for this conclusion but the nature of the evidence is indicated by the fact that mortality after conjugation between individuals recently brought into the laboratory is about 20 per cent to 30 per cent while after conjugation between individuals that have been cultivated many weeks on the standard medium used by Jennings the mortality rises to 94 per cent (Calkins, 1904), or in some of his own cultures from 38 per cent to 59 per cent. If there is such high mortality among ex-conjugants we should expect to find amongst those which were not killed a large percentage of individuals with lowered division-rates, of abnormalities, and an increased variability in these respects, without calling upon incompatible combinations to explain them.

Except for one case of real mutations described below, there is no experimental evidence to indicate that permanent variations in Protozoa arise in any such abrupt manner as Jennings describes. In long-continued cultures changes of organization do occur; such changes are sporadic at first but gradually they predominate until the original character becomes sporadic. This, for example, is the case with one of the morphological features of *Uroleptus mobilis*. In the early series the number of macronuclei was almost uniformly 8 (Fig. 1), but an occasional individual was found with more than 8 (up to 10 or 12). In the later series the number has become almost uniformly 14 to 15. It is impossible to tell when the change occurred as it has been so gradual; inbreeding has been strictly adhered to in every series and each series coming from a single ex-conjugant has shown more or less variation in this respect.

Also there has been a slow but decided change in the extent of rejuvenescence after conjugation. The average division-rate per individual per series for the first sixty days of life of the first 50 series was 15.7 divisions in ten days; for the last 50 series it has fallen to an average of 13.1 divisions per ten days. While this would apparently indicate a weakening of the organization of *Uroleptus*, such is not the case, for along with this change in intensity has gone a change in the length of life in division days. The average length of life of the first 60 series was two hundred and ten days of the last 60 series two hundred and sixty-three days. The relative vitality has not changed although there has been a slight change in the manifestations of that vitality. The initial sixty days is no longer always the period of optimum vitality as was the case originally, but the period of greatest vigor now occurs after from one hundred to one hundred and twenty days and this vigor is retained for a longer stretch of the life cycle (Fig. 232). Such changes are gradual and imperceptible and are demonstrable only by an analysis of a mass of accumulated data.

While the above conclusion is true for *Uroleptus* generally, it does not preclude the possibility of sudden sports or mutations. These might well be expected in a succession of more than 100 fertilizations. While no morphological changes have occurred in this manner some evidence is afforded by abnormally vigorous series and by exceptionally weak series. The former are represented by Series 89, 19 and 45 (see Table, p. 560). The first of these (Series 89) with a relative vitality of 103.6 per cent was the double organism already described (p. 465). This can scarcely be called a mutation although it was a real case of merogamy with double amphinuclei, and the zygote continued to breed true by uniparental inheritance for 367 generations. It never formed cysts and never conjugated but died after four hundred and five days of life. Series 19 with a relative vitality of 110.4 per cent was quite unusual. It lived for five hundred and ninety-eight-division days and divided 597 times and its curve of vitality was of an entirely different type from other series of *Uroleptus* (Fig. 237). It arose as a third generation of a set of old-age conjugations and a relatively low vitality was to be expected. The opposite result must have been due to some combination possibly associated with conjugations of old parents and grandparents. Its peculiarities were not handed on to its progeny (Series 41, 43 and 44). It is possible that Mast's (1917) "mutation" in *Didinium nasutum* and Woodruff's long-lived series of *Spathidium spathula* were analogous cases of unusual germinal combination (Woodruff and Moore, 1924). We do not agree with Woodruff in interpreting our Series 19 as he does his unusual race of *Spathidium* as due to a fortunate aggregate of

suitable but mysterious environmental conditions which were absent in all other series.

Series 45 with a relative vitality of 105.1 per cent was another and a similar case of unusual combinations. Like Series 19 it came from a parental series of low vitality (Series 39, vitality 48.9 per cent) and was exceptional in having a high division-rate over a long period. Its peculiarities were not handed on to its offspring (Series 53).

Other extraordinary combinations resulting in abnormally weak series may also be regarded as incompatible combinations. These are characterized by having both low initial and low general vitality, the most extreme cases coming from parents of extremely old age. They are of no experimental value for they quickly die out (see

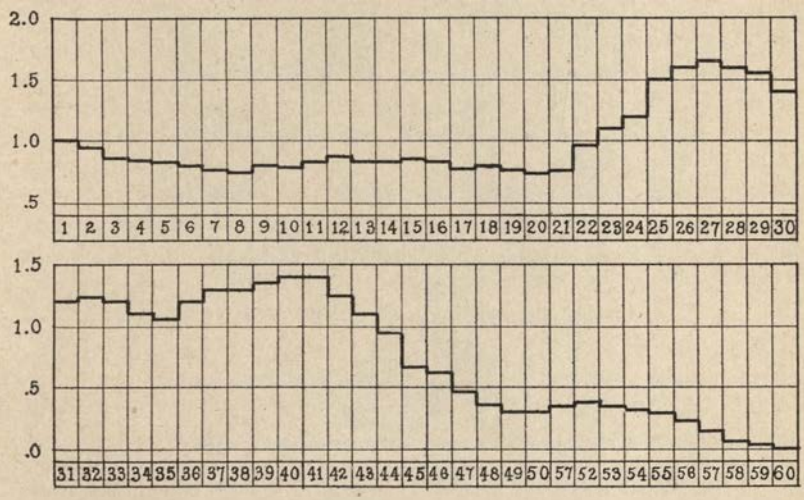

FIG. 237.—*Uroleptus mobilis;* vitality graph of Series 19.

Table, p. 560). It is significant that these exceptional cases all came from old-age individuals with differentiated protoplasm through long-continued metabolic activity and cell division. It appears to be a period when unusual combinations are possible, as with Series 19, 45, etc. It may have been such combinations which resulted in Prandtl's (1906) instances of *Didinium nasutum* in which zygote nuclei develop into macronuclei without differentiation of micronuclei, or of amicronucleate races generally (Patten, 1921).

Apart from the macronuclei, and type of vitality, there is no evidence of a permanent change of the genotype in *Uroleptus mobilis* and there is no evidence at all of permanent mutations, and the same conclusion applies to so-called mutations in Protozoa

generally with the exception of *Chilodon uncinatus* described by MacDougall (1925). The race of *Paramecium* with an increased number of contractile vacuoles described by Hance (1917) may prove to be another example; or changes in organization of *Para-*

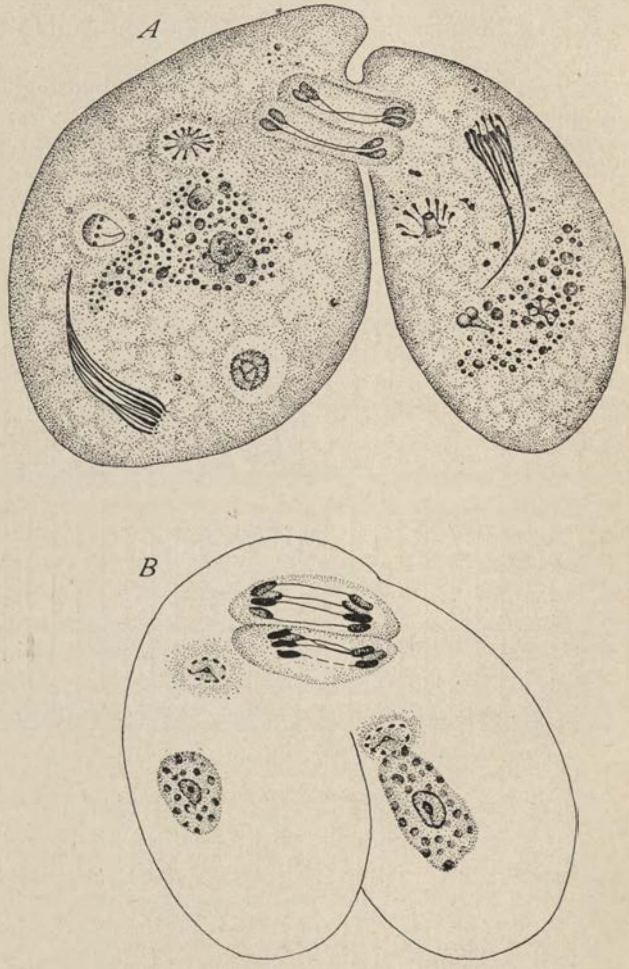

FIG. 238.—*Chilodon uncinatus;* third division and interchange of nuclei of diploid (A) and tetraploid (B) stock. (After MacDougall.)

mecium which were due to heat applied during the "sensitive" period of reorganization after conjugation and which persisted through five successive generations by conjugations, may be still another (Jollos, 1923).

A single individual of *Chilodon uncinatus* was isolated by MacDougall (1925) in December. Its progeny were maintained in pure line cultures until lost in June. In May, larger forms appeared in the cultures and these increased until they out-numbered the smaller forms few of which could then be found. Cytological examination showed that the larger form was morphologically identical with the smaller form, with the exception of the micronuclei in which the chromosomes were eight in number as against four in the smaller form. MacDougall worked out the meiotic divisions for both types and found a similar history in both (Fig. 238) and correctly interprets the tetraploid form as a mutant from the ordinary diploid type.

The Protozoa, finally, cannot be regarded as simple organisms which may be changed in structure or function at will. Each type has a remarkable tenacity of life which we believe is the same as organization and which may be temporarily modified by environmental changes but in which permanent changes are rare and when they occur must come apparently from within. Life or organization on the one hand, is continuous and has been handed down from the indefinite past to the species which we know today through their ancestors. Vitality on the other hand is discontinuous and variable and is manifested by the sum of activities which take place in the organization at any time. Death is not of necessity the cessation of vitality but the disintegration of the organization after which vitality is impossible.

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