POLISH ACADEMY OF SCIENCES
NENCKI INSTITUTE OF EXPERIMENTAL BIOLOGY

PROGRESS IN PROTOZOOLOGY

Proceedings of
VI INTERNATIONAL CONGRESS OF PROTOZOOLOGY
Warszawa, Poland, 5–11 July, 1981

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PART II

Editors:

S. DRYL, S. L. KAZUBSKI, L. KUŹNICKI and J. PŁOSZAJ

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INTRODUCTORY REMARKS

This Part II of the Post-Congress Volume "Progress in Protozoology" of VIth International Congress of Protozoology (5–11 July, 1981, Warsaw, Poland) appears approximately more than one year later than Part I. The main reason for publishing it as two separate parts was a long delay in supplying us with the manuscripts containing the chairpersons summaries of Round-Table Discussion on "Phylogenetic Relationships among Protozoa" and of Symposia which were supplemented with "List of Scientific Reports Presented at Symposia, Contributed Paper and Poster Sessions". Part II of "Progress in Protozoology" contains those summaries which were received by Editors before March 1983; included is also an additional chapter entitled: "International Collaboration Among Protozoologists During the Years 1961 to 1981 (by L. Kuźnicki and B. M. Honigberg), illustrated with numerous photographs carried out during Congresses.

I must state with great regret that not all expected summaries arrived and in consequence the Editors decided in these cases to publish only short notes about the subject of meeting, supplying only information about names of the chairpersons and speakers, who took part in the discussion.

Stanisław DRYL

President, VIth International Congress of Protozoology

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Round Table Discussion

Phylogenetic Relationships among Protozoa

B. M. HONIGBERG

(Chairman and Rapporteur)

Department of Zoology, University of Massachusetts, Amherst, MA 01003, USA

The "Round-Table Discussion" which occupied the entire morning of 15 July, 1981, the last day of the Sixth International Congress of Protozoology, involved a large panel of experts and attracted a very large audience. The primary interests of some of the members of the audience were far removed from the subjects under consideration, yet they seemed to be sufficiently attracted by what was being said that they remained in the lecture hall for a long time. Judging from the amount of discussion among the members of the panel and from the expressions of disappointment from many members of the audience who, because of time limitations, were unable actively to participate in the discussion, it is apparent that similar meetings ought to be planned for the future Congresses. More time, perhaps even an entire day, should be set aside for the consideration of the Phylogeny of Protozoa. In light of the lively discussion that took place during the "Round-Table" conference and in that of the conclusions reached at the meeting, it seems that the publication of "The Newly Revised Classification of the Protozoa" (Levine et al. 1980) might have been somewhat premature. With regard to future schemes, a consensus can best be achieved after much discussion at national and international meetings and after an active exchange of communications among experts from various lands. Such constant exchanges should, I feel, be initiated and encouraged by the Chairman and Members of the standing Committee on Systematics and Evolution of the Society of Protozoologists.

Session took place on July 11, 1981 at VI International Congress of Protozoology, Warsaw, Poland, 5–11 July 1981.

In presiding over the deliberations of the panel of experts, the Chairman was aided by three Honorary Co-Chairmen, Professors P.C.C. Garnham (U.K.), W. Michajłow (Poland), and G.I. Poljansky (U.S.S.R.).

Each higher taxon, depending upon the group being discussed, a PHYLUM, SUBPHYLUM, or SUPERCLASS as defined in the most recent scheme published by the Society of Protozoologists (Levine et al. 1980), was commented upon briefly by one or two speakers and several officially appointed and voluntary discussants from among the members of the panel. Taxa down to, and including SUBORDERS were those primarily considered. In addition, the final part of the Discussion dealt with the question as to whether the Protozoa constitute a "Natural Subkingdom."

Not all taxa were discussed in equal depth, either because not equal bodies of information are available about all of them, or because some of the originally invited speakers and discussants were unable to attend the Congress.

Some speakers submitted complete bibliographies with their contributions; others did not. Because of the great differences among the presentations, no attempt can or will be made to keep the style uniform throughout this report.

Subphylum MASTIGOPHORA Diesing, 1866

Speakers
Prof. J. Mignot (France)
Prof. K. Vickerman (U.K.)

Discussants
Prof. B. M. Honigberg (U.S.A.)
Prof. W. Michajłow (Poland)
Prof. F. J. R. Taylor (Canada)

Class 1. PHYTOMASTIGOPHOREA Calkins, 1909

On the basis of the ideas expressed by numerous investigators (actual references not submitted), including B. B. Bouck, P. Bourrely, G. Brugerolle, J. D. Dodge, H. Ettl, P. Gayral and collaborators, S. Gibbs, J. C. Green, B. Fodt, D. Hibbert, G. F. Leedale, A. R. Loeblich, III, I. Manton, Q. Moestrup, J. D. Pickett-Heaps, F. Schnepf, F. J. R. Taylor, Van Valkenburg, and Wujek, Prof. MIGNOT proposed certain important modifications of the class PHYTOMASTIGOPHOREA as recommended by Levine's Committee (1980). Although in many instances the taxa are the same as those included in Levine et al. (1980), the diagnoses differ, reflecting the approach of Prof. Mignot to the classification.

In his abstract Mignot (1981) stated that in the recent classification of the Society of Protozoologists (Levine et al. 1980) the entire assemblage of PHYTOMASTIGOPHOREA occupies about one page, of which nearly one-half is devoted to EUGLENIDA and only a few lines to DINOFLAGELLIDA. It seems, therefore, that there is an inbalance in treatment of the class with regard to space devoted to the several orders; the degree of attention centered upon certain assemblages is disproportionate to the diversity and numbers of species they contain. Other workers present at the Congress and in subsequent correspondence leveled similar critiques against the Society's scheme. Prof. Mignot thought the inbalance in the space and attention devoted to the ordinal taxa of MASTIGOPHORA was even greater with regard to PHYTOMASTIGOPHOREA *vs* ZOOMASTIGOPHOREA. Actually, many participants of the Panel, and of the Congress in general, felt that there was no justification in separation of the flagellate protozoa into the two classes, and this view will be discussed later in this report.

Another objection of Prof. Mignot (and of some of the other members of the Panel) was the omission of some or many structural and physiological characteristics from the diagnoses of the phytomastigophorean taxa included in the Society's scheme. Among the morphological attributes, he would include the fine structure of the cytoskeleton, and among the physiological ones, the chemical composition of the pigments. All such characters could provide useful criteria for evolutionary considerations.

Much work remains to be done, and meaningful results cannot be achieved without a close collaboration between phycologists and protistologists which has not been realized to date. To provide a basis for a discussion, Prof. Mignot proposed the following scheme for PHYTOMASTIGOPHOREA. The class was to be divided into 11 orders: 1. CHRYSOMONADIDA, 2. PRYMNESIIDA, 3. SILICOFLAGELLIDA, 4. HETEROCHLORIDA, 5. CHLOROMONADIDA, 6. CRYPTOMONADIDA, 7. DINOFLAGELLIDA, 8. SYNDINIIDA, 9. EUGLENIDA, 10. PRASINOMONADIDA, 11. VOLVOCIDA. In turn, the first five orders could be brought together in one class, CHROMOMASTIGOPHOREA Mignot, 1981, or superorder, CHROMOMASTIGOPHORIDEA Mignot, 1981. If this course were followed, CRYPTOMONADIDA, DINOFLAGELLIDA, SYNDINIIDA, and EUGLENIDA would be elevated to the rank of classes or subclasses. The basis for this elevation is to be found in the structural complexity of the representatives of the assemblages which cannot be considered primitive and which differ profoundly from one another in many attributes. The last class, CHLOROMASTIGOPHOREA Mignot, 1981, with clear plant affinities, would include

PRASINOMONADIDA and VOLVOCIDA. In addition, Prof. Mignot (1981) proposed the division of PRYMNESIIDA into three suborders: PRYMNESININA, PAVLOVANINA, ISOCHRYSININA. The order DINOFLAGELLIDA was divided into as many as 15 suborders, which evidently should be discussed in detail before they are accepted; in fact, the author provided no diagnoses for these taxa, referring merely to the authorities of each of them. As far as the class EUGLENOMASTIGOPHOREA (or super-order EUGLENOMASTIGOPHORIDEA) is concerned, it was to include a single order EUGLENIDA Bütschli, 1884 and four suborders: EUTREPTIINA Leedale, 1967; EUGLENINA Bütschli, 1884; RHABDOMONADINA Leedale, 1967; and TERONEMATINA Leedale, 1967. It should be noted that Prof. Mignot found unacceptable the separation between EUTREPTIINA from EUGLENAMORPHINA Leedale, 1967 and RHABDOMONADINA from SPHENOMONADINA Leedale, 1967.

A summary such as this is not an appropriate place to publish a new classification scheme. Yet to show the differences between the kind of diagnosis proposed in many instances by Prof. Mignot and that employed by the Committee (Levine et al. 1980), I shall cite below the diagnoses of CHRYSOMONADIDA given by him and by the Committee.

Committee of the Society of Protozoologists (Levine et al. 1980), CHRYSOMONADIDA Engler, 1898. Two unequal flagella, one directed anteriorly and bearing 2 opposite rows of mastigonemes, other trailing and smooth; chloroplast golden-brown or absent; storage products chrysolaminarin and fat; cell naked, with richly patterned silicified scales, or with lorica; sexual reproduction present.

Dinobryon, Ochromonas, Synura

Mignot 1981 (presentation at the Congress), CHRYSOMONADIDA Engler, 1898 emend. Hibberd, 1976. — Two flagella, one directed anteriorly, bearing 2 opposite rows of tubular complex mastigonemes; zone of transition helicoidal; flagellum short, inflated at its base; cytoskeleton consisting mainly of 2 microtubular cortical systems, 1 formed by microtubules radiating from a fibrillar MTOC, situated at the dorsal edge, and by a rhizoplast associated with the nucleus; yellow-brown chloroplasts contain chlorophylls *a* and *c*, β-carotene, lutein, fucoxanthin, didinoxanthin, violaxanthin; plastid lamellae formed by 3 thylokoids of which the peripheral ones are circular; stigma in plastid or leucoplast; single dictyosome surrounding nucleus; polysaccharide reserve in vacuoles is chrysolaminarin; cells naked or covered with silicified scales of specific complex structure; or in lorica of organic material (cellulose?); formation of scales in intracellular silicalemma originating in Golgi complex associated with endoplasmic reticulum, fre-

quently periplastidial; encystment by endogenous formation of silicified cyst wall with specific structure, pore, and aperture; very marked polymorphism with development of microfibrillar part of cytoskeleton into rhizopodial forms.

Ochromonas, Dinobryon, Synura

It should be added here that in the diagnosis of the class or superorder CHROMOMASTIGOPHOREA (IDEA) Prof. Mignot specified also that the mitochondria have ampouliform (inflated) or tubular cristae.

Opinion has been expressed by several members of the Panel that the foregoing, and some of the other diagnoses given by Prof. Mignot tend to be too long and detailed. It cannot be denied, however, that they are very complete.

There were certain praises as well as criticisms voiced during the session with regard to the Society's scheme of classification (Levine et al. 1980) as well as with regard to that of Prof. Mignot. It was pointed out by Prof. Taylor that the Mignot scheme represents a beginning toward recognition of natural relationships among the flagellates. He considered CHROMOMASTIGOPHOREA as a good, natural grouping. On the other hand, he felt that separation of VOLVOCIDA from their very close relatives Chlorococcales was unnatural. Prof. Taylor not only further emphasized the need for a closer collaboration between phycologists and protozoologists, but felt that such a collaboration must include also the mycologists.

Class 2. ZOOMASTIGOPHOREA Calkins, 1909

As far as the separation of PHYTOMASTIGOPHOREA and ZOOMASTIGOPHOREA is concerned, several members of the Panel, including Prof. Taylor, felt that it is unnatural and should be eliminated. The proposed elimination of these two classes while undoubtedly sound — it would not separate what appear to be related assemblages at a class level — must be the subject of further discussion. It has been suggested, for example, that a new scheme should involve elevating some groups from the taxonomic levels which they now occupy. Prof. Taylor went so far as to suggest that the dinoflagellates, with a diversity and distinctness resembling that of CILIOPHORA, might actually deserve the rank of a subphylum. A number of questions will have to be answered, as the result of ongoing discussions, before we can make major changes in the admittedly artificial class ZOOMASTIGOPHOREA. Among these are the following: 1. Are there to be no

superorders among the nonpigmented flagellates, save PARABASALIDEA? 2. Is there enough evidence in support of the kinships suggested by Brugerolle (1977) for RETORTAMONADIDA, DIPLOMONADIDA, and OXYMONADIDA to allow the establishment of superorder (class?) for these orders? 3. Where actually do CHOANOFLAGELLIDA and BICOSOECIDA belong in the general classification scheme; could they be placed in a higher taxon? 4. Is there any justification for dividing the nonpigmented flagellates into the somewhat informal superordinal groupings recommended by Grassé (1952)?

Although for convenience's sake (we still have difficulty with avoiding this pragmatic concept), we shall discuss here the orders of ZOOMASTIGOPHOREA, as was done by Prof. VICKERMAN, we ought not to lose sight of the need for reconsidering the validity of this class as well as of PHYTOMASTIGOPHOREA. As I suggested during the Round-Table Discussion, these taxa may well be soon put to rest.

Professor Vickerman concentrated on the changes in the orders of ZOOMASTIGOPHOREA between the first (Honigberg et al. 1964) and second (Levine et al. 1980) Systems of Classification published by the Society of Protozoologists. He noted that these changes were more noticeable among the "lower" mostly free-living nonpigmented flagellates than among the structurally more complex and almost exclusively parasitic forms. He felt that the problems of classification and evolution of KINETOPLASTIDA Honigberg, 1963, DIPLOMONADIDA Wenyon, 1926 emend. Brugerolle, 1975, and TRICHOMONADIDA Kirby, 1947, emend. Honigberg, in Camp, Mattern and Honigberg, 1974, which were discussed at the Fourth International Congress of Parasitology in Warsaw, 1979 (Honigberg et al. 1982), could be left out of the present consideration. He also did not discuss RETORTAMONADIDA, analyzed in some detail by Kulda and Nohýnková (1978), and PROTEROMONADIDA Grassé, 1952 emend. Vickerman, 1976, which he as well as Kulda and Nohýnková (1978) considered as quite separate from KINETOPLASTIDA. Professor Vickerman felt, however, that more ultrastructural details are needed to enable us to obtain an understanding (of OXYMONADIDA and HYPERMASTIGIDA) comparable to that we have now of kinetoplastids, proteromonads, retortamonads, diplomonads, and trichomonads. It is a pity that Dr. Brugerolle who did much work on the fine structure of the last two groups could not be present, for he could have discussed the relationships among RETORTAMONADIDA, DIPLOMONADIDA, and OXYMONADIDA as suggested in his 1977 publication.

Among the problems discussed by Prof. Vickerman was that of CHOANOFLAGELLIDA. These organisms, with a single flagellum and a collar made up of a ring of tentacles, were at one time considered as

algae by phycologists (Bourelly 1968, Christensen 1962, 1966). Subsequently, however, on the basis of electron-microscopic studies (Hibberd 1975, Leadbetter 1972, Leadbetter and Mantow 1974), they were removed from among the plants and by implication from PHYTOMASTIGOPHOREA. Although the choanoflagellates share certain structural characteristics with the choanocytes of sponges and of other metazoa (Norrevang and Wingstrand 1970), their retention in a separate protozoan order appears entirely justified. No one took issue with this assertion.

The question of BICOSOECIDA Grassé et Deflandre, 1952, appears more complex. Hall (1953), among others, placed these organisms among the chrysomonads, and more recently Mignot (1974) assigned *Bicoeca* to this assemblage of pigmented flagellates. He thought that this group of unpigmented heterokont, lorica-dwelling flagellates might be appended to the *Chrysophyceae,* but, according to Vickerman, a number of features sets *Bicoeca* apart from these algae. Among the structures in question is the long tongue-like appendage (languette) which arises alongside the two flagella and is supported by a row of microtubules. Although the languette is somewhat reminiscent of the heptonema found in *Prymnesiophyceae,* in *Bicoeca* it is involved in food capture. Although the mastigonemes on the anterior coiling flagellum of BICOSOECIDA are tubular and similar to those found in CHRYSOMONADIDA, the anchoring recurrent flagellum, having a fibrillar sheet and attachment cone is distinctive. It must be also remembered that the carbohydrate storage product of the bicosoecids is glycogen and not leucosin. In a subsequent personal communication to me, Prof. Vickerman stated with regard to BICOSOECIDA: "I do not think they should be in a separate order if *Silicoflagellida* (in Honigberg et al. 1964; Levine et al. 1980) and *Ebriida* (in Honigberg et al. 1964) and other chrysomonad appendages should. There are certainly enough ultrastructural features to set them apart."

The unnatural and clearly polyphylectic nature of the order RHIZOMASTIGIDA Doflein, 1916 was pointed out by the committee of the Society of Protozoologists which was responsible for the 1964 classification scheme. At that time Honigberg and Balamuth stated in a footnote: "The presence of simple mastigonts in trophic stages is the basis for alignment with *Mastigophora* rather than with *Sarcodina*, while at the same time the concomitant occurrence of flagella and pseudopodia strikingly illustrates the basic affinities of generalized flagellates and amebae (as originally suggested by Pascher 1918). The tendency for secondary reduction of flagella is observed in numerous representatives (of the order). The polyphylectic nature of the group is indicated by the presence of at least two distinct kinds of pseudopodial organiza-

tion among different members. One pattern (exemplified by *Naegleria*) is characterized by the lobose pseudopodia encountered in amebae of the subclass *Lobosia*. Another pattern (exemplified by *Dimorpha* and other helioflagellates) is marked by typical axopodia terminating internally in a central granule or centroplast, as in heliozoa of the order *Centrohelida*. Future studies can be expected to lead to a more natural regrouping of the forms placed here for convenience." As might have been expected, the order RHIZOMASTIGIDA was omitted from the more recent scheme published by Levine et al. (1980). Actually, the constitution of this "order" has varied among the standard protozoology texts; e.g., Hall (1953) included the following genera among RHIZOMASTIGIDA: *Histomonas, Heliobodo, Mastigamoeba, Mastigella, Mastigina* and *Rhizomastix*. Amon other workers, he felt also that *Pteridomonas* was a chrysomonad and that *Actinomonas* and *Dimorpha* had helioflagellate affinities. Grassé (1952) thought that *Pteridomonas* was also a helioflagellate. Many authors (e.g., Kudo 1966) included *Tetramitus* and *Naegleria* in RHIZOMASTIGIDA. Certain protozoologists assigned at least some of the rhizomastigid genera to many flagellate and rhizopod assemblages. Vickerman, who until recently (Vickerman 1976) considered *Cercomonas* and *Heteromita* as BODONINA *incertae sedis*, included these two genera in his discussion of zooflagellates at the 1981 session in Warsaw. According to him (Vickerman, personal communication): *Cercomonas* and *Heteromita* are clearly related to each other and sufficiently distinct from both chrysomonads and sarcodines to merit a separate small order. Accordingly, using the characteristics presented by him at the 1981 Congress, I propose to create, with Vickerman as the sole authority, a new order among MASTIGOPHORA.

Order CERCOMONADIDA Vickerman

Two heterodynamic acronematic flagella whose kinetosomes cap cone of microtubules closely enveloping drawn out anterior part of nucleus; cone apparently attached to nucleus; microtubules, some lying along nucleus, not all subpellicular (as in *Kinetoplastida*); single, membrane-bounded, postnuclear organelle of unknown function loosely capping posterior part of nucleus; extrusomes resembling haptocysts of heliozoans often associated with surface microtubule tracks; several mitochondria with tubular cristae; food ingestion by pseudopodia; contractile vacuole usually postnuclear, may be adbasal (as in *Kinetoplastida*).

Cercomonas, Heteromita

Some of the former members of RHIZOMASTIGIDA can be now assigned to known flagellate and rhizopod taxa, and this is reflected in

the 1980 classification of Levine's Committee. Thus *Histomonas* and *Dientamoeba* (see Honigberg and Bennett 1971, Honigberg and Kuldová 1969, Honigberg in Camp et al. 1974) are considered as modified trichomonad genera (order TRICHOMONADIDA); *Tetramitus* and *Naegleria* have been included in the rhizopod order SCHIZOPYRENIDA Singh, 1952.

According to Vickerman, in certain respects *Mastigamoeba* resembles the cercomonads. It possesses the prenuclear cone of microtubules, pseudopodia, and similar mitochondria, but it lacks the postnuclear organelle and the extrusomes; furthermore, there is no recurrent flagellum but only a barren kinetosome corresponding to the basal body of this flagellum. Evidently, *Mastigamoeba* has structural resemblance to the myxamebae of some EUMYCETOZOEA. In a personal communication to me, Vickerman wrote: "Mastigamoeba bears such a close superficial resemblance to slime-mold flagellates that I cultured one of the latter believing it to be *Mastigamoeba* for several years (as cyst, amoeba and flagellate) — till one day it produced beautiful plasmodia." Vickerman wondered if, indeed, the affinities of mastigamoebae lie with mycetozoan sarcodines rather than with any known flagellate group. He stated, however, in the course of the discussion at the Warsaw Congress that a post-nuclear organelle was found in *Rhipidodendron* and *Spongomonas*, both sedentary colorless flagellates with homodynamic flagella (Hibberd 1976). "Is it possible (he asked) that heterodynamism of the two flagella has been lost as a result of adoption of the sedentary habits?"

With regard to another genus, *Mastigella*, which used to be included in RHIZOMASTIGIDA, Vickerman stated that two species of the genus, *Mastigella vitraea* and *Mastigella hylae*, appear quite different from the species of *Mastigamoeba*. Since nothing is known about the fine structure of *Mastigella vitraea*, its assignment to any existing or to a new taxon is not possible at this time. On the other hand, it is known that *Mastigella hylae* is multinucleate, lacks mitochondria, and has cones of microtubules associated with its flagellar kinetosomes. According to unpublished studies of Brugerolle, there are several noncontractile flagella, each associated with one of the nuclei. In view of this, Vickerman believes that *Mastigella* is a "higher" zooflagellate, but appears to have no obvious affinities to any particular group. Perhaps, after more is known about other members of this genus, a new order might have to be established for them.

Since nothing is known about the fine-structural details of many of the other former members of the order RHIZOMASTIGIDA, e.g., *Heliobodo*, *Actinomonas*, *Dimorpha* and *Pteridomonas*, neither Vickerman nor I is ready to place them in any known protozoan as-

semblage or establish new ones for these genera. Thus there still remains a group of former rhizomastigotes which will have to be considered for some time as species *incertae sedis*.

It has been pointed out by Vickerman that one of the problems in placing "lower" zooflagellates in an order is how minimal a definition of the order is acceptable. A good example of this problem quoted by him is the assignment by Hollande (1980) of *Perkinsiella*, the endobiont of the parasitic amebae *Paramoeba* and *Janickina* to KINETOPLASTIDA. *Perkinsiella* has, indeed, a single mitochondrion rich in DNA fibrils which, however, are isotropic in their arrangement. On the basis of the available data, Vickerman concluded: "In the absence of any sign of a flagellar apparatus or pellicular microtubules and of information on nuclear division, the writer would hesitate to assign this organism to KINETOPLASTIDA, though it is possible that adaptation of *Perkinsiella* to existence as an organelle of its amoeba host may have robbed it of most of its kinetoplastid characteristics." Although Hollande (1980) claimed that: "... les microtubules situés à la périphérie du cinétoplaste pourraient être homologues de ceux qui, dans une cellule de *Cryptobia* sont sous-jacents à la pellicule;..." one would tend to concur with Vickerman that the evidence for the presence of subpellicular microtubules is not compelling. In general, although an admirer of Prof. Hollande's cytologic researches, I cannot help but feel that assignments of flagellates or any other protozoa to given taxa ought to be based on stronger evidence than that provided by him for *Perkinsiella*.

It is evident from the foregoing discussion that although much is known about both the pigmented and nonpigmented flagellate groups, which constitute the basic assemblages of the greatest importance to evolution of eukaryotes, there remain many important problems still to be solved. Clearly the solution of some of these problems will depend upon mutual understanding and effective collaboration among protozoologists, phycologists, and mycologists.

Appendix to MASTIGOPHORA

Prof. W. Michajłow presented his views on the taxonomy and taxonomic criteria that ought to be employed for the group of parasitic euglenoids "*Euglenida parasitica*," which he has been studying for many years. Since we are concerned in the present discussion primarily with higher taxa, we cannot consider details of taxa below suborders. It suffices to say that in classifying the parasitic euglenids, Michajłow wishes to employ the following criteria: 1. structure of parasitic and free-living forms (present in the life cycle); 2. details of the developmental cycles;

3. the type of movement characteristic of the parasitic and free-living forms; 4. host specificity based upon statistical data; 5. geographic distribution.

The editors of the Post-Congress Publication may wish to include the entire presentation in an Appendix to the present discussion (see p. 219).

Superclass RHIZOPODA von Siebold, 1845

Speaker
Dr. T. K. Sawyer (U.S.A.)

Discussants
Prof. J. B. Jadin (Belgium)
Prof. J. J. Lee (U.S.A.)

Dr. Sawyer did not transmit to us the full text of his remarks and there was relatively little discussion of the very important superclass RHIZOPODA, probably because several of the experts concerned with this group were unable to attend the Congress. Consequently, the reader is referred to the abstract published by Dr. Sawyer in Progress in Protozoology, the Congress Proceedings, which were distributed among the participants at the registration desk in Warsaw.

The only person who made extensive remarks in connection with RHIZOPODA was Prof. J. B. Jadin. His most interesting presentation was entitled "Relation entre protozoaires, bactéries et virus."

Superclass ACTINOPODA Calkins, 1909

Speaker
Prof. C. Bardele (Fed. Rep. Germany)

Discussants
Prof. J. J. Lee (U.S.A.)
Dr. D. J. Patterson (U.K.)

Prof. Bardele expressed his full agreement with the views of the absent Dr. Merinfeld (see Merinfeld 1981) that ACTINOPODA do not represent a natural assemblage, but rather have to be regarded as an artificial taxon. According to Prof. Bardele, only those species presently available for cytologic analysis by modern methods should be employed in all future considerations of the organisms now included among ACTINOPODA. The species that have not been located and examined since the turn of the century ought to be excluded from such considerations.

The speaker listed a series of characteristics he considers useful in the analysis of phylogenetic relationships among the actinopods: (a) asexual reproduction — binary or multiple fission; (b) location of the division spindle and the type of polar bodies present during mitosis; (c) sex-

ual reproduction — present or absent; (d) flagellated stages — present or absent; (e) flagellum naked or with mastigonemes; (f) uni-*vs* multinucleate condition; (g) pattern of axonemal microtubules — absent, triangular, hexagonal, hexagonal and triangular, double spiralled, dodecahedral with or without peripheral spirals; (h) MIOC associated with a nuclear envelope, an axoplast, or a centroplast with a tripartite disc; (i) motility mechanisms; (j) type of extrusomes, muciferous bodies, or kinetocysts; (k) mitochondria entering or not entering the axopodia; (l) skeleton consisting of one to many pieces; (m) skeleton — intra- or extracellular; (n) material of which the skeleton is made — organic, silica, strontium sulfate, calcium carbonate; (o) capsular membrane location — intra- *vs* extracellular; (p) capsular membrane — material of which it is made and symmetry; (q) life cycle with or without metamorphosis; (r) habitat — marine, brackish, fresh-water; (s) mode of life — planctonic, benthic, attached to substrate (stalked).

If the aforementioned attributes, a part of which can be observed only by electron microscopy, are considered, it becomes evident that neither ACTINOPODA nor the various sub-groups of this superclass, e.g., the former RADIOLARIA Müller 1858, as listed in Honigberg et al. (1964) (present POLYCYSTINEA Ehrenberg, 1838 and PHAEODAREA Haeckel, 1879 as listed in Levine et al. (1980)), HELIOZOEA Haeckel, 1866, or CENTROHELIDA Kühn, 1926, are monophylectic. All these designations, irrespective of their taxonomic level, could be maintained for teaching purposes. It might even be advisable to style these names in lower case, thus avoiding any taxonomic implications. If, however, as is the usual custom, the names such as ACTINOPODA or HELIOZOEA are employed for designation of definite taxa, this should be done with the clear understanding (stated in writing) that these groups appear to be polyphyletic and are the reflection of the present imperfect knowledge of many species.

Since only a few species representative of the 13 orders belonging to the three classes of "radiolarians," ACANTAREA, POLYCYSTINEA, and PHAEODAREA, listed in the recent classification system of the Society of Protozoologists (Levine et al. 1980), have been studied sufficiently at the fine-structural level, Prof. Bardele limited his subsequent comments to the heliozoa.

While Merinfeld (1981) divides the heliozoa into six natural "ultrastructural clusters," Bardele (in Cachon and Balamuth 1977) recognizes seven groups at the family level, i.e., *Ciliophryidae, Clathrulinidae, Actinophryidae, Taxopodidae, Acanthocystidae, Heterophyidae,* and *Gymnosphaeridae*. Grouping these families into orders is still debatable. For example, Davidson (1974) considers *Ciliophryidae* (the helioflagellates) as closely related to chrysomonads. On the other hand, Febre-Chevalier (personal communication) has created a new

heliozoan order CILIOPHRYIDA for the same organisms. The phylogenetic relationships of *Clathrulidae*, *Actinophryidae*, and especially of *Taxopodidae* to the remaining heliozoan families is unknown. In view of this, it might be advisable to elevate these families to the ordinal rank. While *Acanthocystidae* and *Heterophryidae* are closely related to each other, they appear to have little if any kinships with *Actinophryidae*. According to Bardele (1977), the former two groups, together with the third centrohelidian family *Gymnosphaeridae*, might be more closely related to the radiolarian assemblages than to the other heliozoa.

One of the discussants, Dr. Patterson, agreed in general with the views expressed by the speaker. Furthermore, he emphasized the need for detailed studies of the flagellated stages wherever they occur in the life cycles of the actinopod species. Patterson suggested that there might have been two evolutionary lines among the actinopods:

1. lobose amebae → filose amebae → heliozoa → other actinopods and

2. chrysomonads → pedinellids → helioflagellates → heliozoa.

All other problems notwithstanding, the actinopod groups clearly belong among SARCODINA.

Phylum APICOMPLEXA

Speakers	Discussants
Prof. S. Desser (Canada)	Dr. Tamara Beyer (U.S.S.R.)
Prof. E. Scholtyseck (Fed. Rep. Germany)	Prof. P. C. C. Garnham (U.K.)
	Prof. M. V. Krylov (U.S.S.R.)
	Prof. E. Vivier (France)

The lively discussion of APICOMPLEXA occupied much time. Since the speakers discussed the entire phylum and did not limit themselves to specific groups, I prefer to deal with each presentation separately rather than to combine them.

Prof. SCHOLTYSECK initiated the discussion on APICOMPLEXA. According to him, the principles to be used in systematics of Protozoa should be concerned primarily with morphological aspects, including fine-structure. The latter constituted the basis for the creation of the phylum APICOMPLEXA by Levine (1970). The speaker illustrated the basic structural characteristics by a schematic diagram of a coccidian merozoite (Fig. 1). It includes: the trilaminar pellicle (PE), polar rings (P), micropore (MP), conoid (C), rhoptries (RH), and micronemes (MN). All these structures are located mainly in the apical region of the motile stages. In light of the validity of the aforementioned morphological characteristics in the differentiation of the members of the phylum from all the remaining protozoan assemblages, the relatively recent name APICOMPLEXA has been accepted by most protozoologists.

On phylogenetic grounds, Prof. Scholtyseck suggested a divison of the phylum into two subphyla, SPOROZOA and PIROPLASMA.

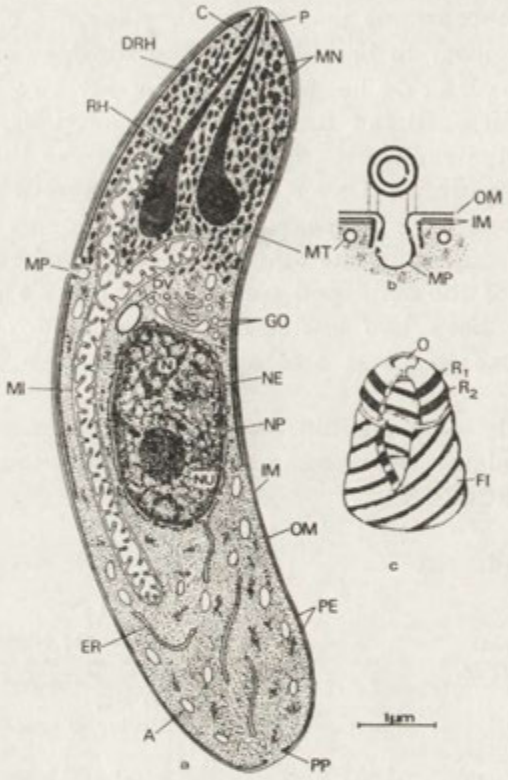

Fig. 1. Schematic diagram of a coccidian merozoite showing the diagnostic fine-structural characteristics. (a) longitudinal section of the organism, (b) longitudinal and transverse sections of a micropore, (c) a diagram of a conoid. A — amylopectin, C — conoid, DRH — ductule of rhoptry, ER — endoplasmic reticulum, FI — fibril, GO — Golgi apparatus, IM — inner membrane complex, MI — mitochondrion, MN — micronemes, MP — micropore, MT — mirotubules, N — nucleus, NE — nuclear envelope, NP — nuclear pore, O — opening, OM — outer membrane, P — polar ring, PE — pellicle, PP — posterior polar ring, R_1, R_2 — preconoidal rings, RH — rhoptry

In their life cycle the organisms belonging to the former subphylum have an encysted zygote, sporocysts in the gregarines and oocysts in the coccidia. Meiosis and sporogony occur in these stages. Therefore, it would be useful to preserve the historical name SPOROZOA Leuckart, 1879 for this subphylum.

Since to date the occurrence of gamogony among piroplasms has not been unequivocally proved, this group should be separated from SPOROZOA and placed in a new subphylum PIROPLASMA.

The foregoing subdivisions of APICOMPLEXA reflect Prof. Scholtyseck's belief that asexual reproduction, i.e., schizogony (or merogony), represents a secondary development in the course of evolution of the phylum found only in the more advanced intracellular SPOROZOA. The more primitive gregarines and coccidia have only the sexual reproductive phase.

According to the speaker, if one follows his approach to the taxonomy of APICOMPLEXA, the genus *Perkinsus* Levine, 1978, the sole member of the class PERKINSEA Levine, 1978, is to be considered not as sporozoan, but as an apicomplexan. On the other hand, the members of the order AGAMOCOCCIDIIDA Levine, 1979 do not belong among COCCIDIA Leuckart, 1879.

A different approach to APICOMPLEXA was presented by the second speaker, Prof. DESSER.

It was his understanding that the name APICOMPLEXA was created by Levine (1970) to replace the older and generally employed name SPOROZOA which included only the gregarines, coccidia, and piroplasms. In addition to possessing the apical complex, as revealed by electron microscopy, the majority of the species belonging to APICOMPLEXA have life cycles that involve gamogony and sporogony; in most instances they include also merogony.

The inclusion of the class PERKINSEA Levine, 1978 among APICOMPLEXA (with the single genus *Perkinsus* Levine, 1978) in the most recent scheme published by the Society of Protozoologists (Levine et al., 1980) is not justified. Admittedly, the zoospores of this peculiar organism possess certain features which superficially resemble the apical complex. These features might have resulted, however, from convergent evolution of organisms of different ancestry and function in a similar manner to enable the parasites to assume their intracellular location. Moreover, these parasites do not undergo sexual reproduction, sporogony, or merogony. Finally, the zoospores of *Perkinsus* sp. (which are not sexual stages) are biflagellate (Perkins 1976). For those reasons, he felt that the class *Perkinsea* should be removed from the APICOMPLEXA and that the original concept of APICOMPLEXA as the equivalent of SPOROZOA, be retained.

Prof. Desser felt that not enough information is available at this time to justify definitive decisions with regard to the affinities of the piroplasms. One must agree with him that much evidence was provided during the Sixth Congress in support of this opinion. One of the lines of evidence was the demonstration by Prof. Vivier that *Dactylosoma ranarum* is an eimeriid coccidian rather than a piroplasm. Intraerythrocytic merozoites of these species have a trilaminate pellicle, po-

lar rings, micronemes, and a conoid; also, centrioles were seen associated with dividing nuclei.

Sexual "behavior" is an important characteristic in ascertaining the taxonomic position of an organism in the subclass COCCIDIA. Therefore, the elucidation of a presumed sexual phase in the life cycle(s) of piroplasms is of major importance. Mehlhorn et al. (1980) indicated that the piroplasms have many characteristics in common with the members of HAEMOSPORINA and that the two assemblages should be considered as "sister groups." According to Maltman (at the present Congress), however his fine-structural observations of a species of *Babesia* cultivated *in vitro* revealed apparent syngamy of gametes, a process unlike that typical of HAEMOSPORINA, but resembling fertilization described from several ADELINA. Furthermore, according to Prof. Desser, the kinetes of the species belonging to the genera *Babesia* and *Theileria* are as similar to the sporokinetes of *Karyolysus* spp. as to the ookinetes of HAMEOSPORINA. The picture is further confused by the study of *Theileria annulata* (Schein et al. 1975) in which the sexual reproduction is said to involve anisogamy, with filariform microgametes. Some workers still doubt the occurrence of sexual reproduction among the piroplasms.

In light of the available data, Prof. Desser concluded that a definite commitment on the taxonomic position of the piroplasms among the APICOMPLEXA should be postponed until the problems outlined in his presentation have been resolved.

Remarks of the Discussants

Dr. Tamara Beyer and Profs. Garnham and Krylov made remarks about APICOMPLEXA.

The following statement was made by Prof. GARNHAM. The two classification schemes published by the Society of Protozoologists (Honigberg et al. 1964; Levine et al. 1980) were concerned only with the higher taxa, down to suborders, but the interrelationships can be more precisely visualized in the taxa below suborders — the former represents concepts, the latter actual organisms. In view of this, he proposed that a special Round Table Discussion be arranged at some future time, for example at the next Congress.

The "phylum" APICOMPLEXA, divided into three "sub-classes," seemed to him natural and fairly neatly to fit into the old term SPOROZOA which we reluctantly abandoned; but electron microscopy has revolutionized the territory and has necessitated a new look. APICOMPLEXA are a unique group in: (a) being obligatorily parasitic and (b) possessing ultramicroscopic structures at the anterior end and a micro-

pore near the center. These and other structures regress at different stages of development and in the degree of juvenility in phylogeny. They are best seen in sporozoites, the product of the sexual phase, and function particularly in the invasion of a host cell, which is an important feature of parasitism.

Three factors are of importance in the phylogeny of APICOMPLEXA: 1. The Stage: characteristic organelles may be present at one stage but absent in another (e.g., tubular mitochondria, cf. Vivier); 2. The Host: It is essential to consider the phylogeny of both vertebrate and invertebrate hosts in heteroxenous examples, as this does not necessarily run parallel with the phylogeny of the parasite — unfortunately there are few indications as to the date when the parasite became established; 3. Zoogeography. The presence or absence of certain parasites in various vertebrate groups in different regions of the world may be linked with major geological events such as continental drift in more remote ages and glaciation in more recent. Factors 2 and 3 are not directly related to classification, but undoubtedly shed light on phylogeny. The intervention of a biting arthropod has long been suggested as the path taken in the phylogeny of haemosporidia from coccidia and of certain haemogregarines from adeleids.

According to Prof. Garnham, the question of free-living ancestors of APICOMPLEXA has assumed new importance in light of the recent observations on *Acanthamoeba* and *Naegleria*, which have illustrated how the host barrier in parasitism can be surmounted. However, ideas about their origin remain highly speculative. We look for three primary characters — a degree of "amoebicity," a flagellate stage, and some indication of sexuality. The members of the suborder *Bodonina* Hollande, 1952 emend. Vickerman, 1976 have been suggested as the possible ancestors of APICOMPLEXA. However, Prof. Garnham's preference is for RHIZOPODA von Siebold, 1875, some members of which possess at least two of these characters, e.g., *Naegleria* in the order SCHIZOPYRENIDA. The "monopodial" cylinder found in some members of this group resembles the structure of the motile zygote (ookinete). The best secondary clues are offered by the sexual stages, e.g., the microgamete and the zygote, which certainly are of immense significance in the more recent phylogeny. Prof. Garnham was still uncertain as to how much help will be obtained through the employment of biochemical features, e.g., isoenzymes and nucleic acid series.

Dr. BEYER was the second discussant. She found no justification for the doubts expressed by some workers with regard to the validity of the name APICOMPLEXA, and adduced the following arguments in support of her viewpoint:

1. Morphological, especially fine-structural, features are, as indicated by Prof. Scholtyseck, the principal bases for separation of the higher protozoan taxa. It is the presence of the apical complex that helps in placing a newly discovered organism in the phylum APICOMPLEXA. This structural characteristic can be recognized rather quickly. On the other hand, finding all stages of a life cycle might take months, years, or, indeed a lifetime of an investigator;

2. It is now generally accepted that the main, or even the only structural pattern found among the members of the phylum APICOMPLEXA is the one characteristic of the class SPOROZOA Leuckart, 1879, or even of the suborder EIMERIINA Léger, 1911. This limited view can be seen in the diagrams of the apical complex included in the majority of the more recent reports. The presence or absence of the conoid or the number and position of rhoptries and of subpellicular microtubules are the features often used for separation of the members of the phylum under consideration. However, this approach is not rational. The structural organization may vary far more, e.g., *Dermocystidium marinum* or *Spiromonas*. Yet the presence of a trilaminar pellicle, or a micropore, or rhoptries etc. can be considered sufficient for inclusion of an organism among APICOMPLEXA, even though any of these structures by itself may not constitute sufficient grounds for assigning a protozoon to SPOROZOA;

3. Dissimilarity in life cycles of organisms having similar structure does not preclude their assignment to APICOMPLEXA. For example, until the late 1960's or early 1970's the life cycles of genera such as *Toxoplasma*, *Sarcocystis*, or *Besnoitia* were considered as distinct from those typical of SPOROZOA. The more recent fine-structural findings indicated the need for inclusion of these genera in APICOMPLEXA, albeit not in SPOROZOA. Subsequent studies of their life cycles showed that *Toxoplasma* and the related genera belonged together with *Eimeria*, *Isospora*, and *Plasmodium* in the order EUCOCCIDIIDA Léger et Dubosq, 1910; they are considered now as members of the suborder EIMERIINA Léger, 1911.

According to Dr. Beyer, the situation cited by her for *Toxoplasma* may apply also to *Dermocystidium* (= *Perkinsus*) *marinum*, the life cycle of which is incompletely understood. Thus, it is impossible to include the latter genus in SPOROZOA. However, the presence of apical structures in *Perkinsus*, although strikingly different from those of "typical" coccidia, provides a sufficient reason for placing this organism in APICOMPLEXA. Further studies are needed before a definite position in this phylum is found for the heretofore neglected genus *Perkinsus*. The discussant expressed no opinion about placing this genus in Levine's (1978) class PERKINSEA. (It should be noted here that several of the

panel members supported Prof. Desser's view-point with regard to Levine's class. They agreed with the former that there are problems even with placing *Perkinsus* in APICOMPLEXA.)

As far as the piroplasmids are concerned, Dr. Beyer, like the previous speakers, emphasized the fact that until now there has been found much more similarity between the piroplasmids and haemosporidians in their ultrastructure than in their life cycles. She also supported Prof. Scholtyseck in separating the piroplasms from SPOROZOA.

The last discussant was Prof. KRYLOV who submitted the following remarks on, and scheme of classification of the phylum SPOROZOA Leuckart, 1879 emend. Krylov et Dobrovolsky, 1980 (he does not accept the name APICOMPLEXA Levine, 1970). His presentation was taken from the chapter which he and A. A. Dobrovolsky contributed to the book on higher taxa of Protozoa (see below).

According to the discussant, SPOROZOA have the following structural characters: (a) trilaminar pellicle in "migrating stages" (merozoites or sporozoites); (b) ultracytostome (micropore); (c) polar rings; (d) subpellicular microtubules; (e) conoid; (f) rhoptries and micronemes. According to the principles formulated by Cuvier as early as in 1817, SPOROZOA, with a common plan of organization, should be placed in a separate animal phylum. Within the limits of this phylum Prof. Krylov recognizes three large groups:

1. Class PERKINSEMORPHA Levine, 1978 emend. Krylov, 1980. Orders with features characteristic of all sporozoans; with flagellated vegetative stages;
2. Class GREGARINOMORPHA Dufour, 1928 emend. Krylov et Dobrovolsky, 1980.
   Gametogenesis similar in both sexes;
3. Class COCCIDIOMORPHA Doflein, 1901 emend. Krylov, 1980
   Gametogenesis usually different in male and female gametes.

The discussant believes that the class COCCIDIOMORPHA includes two phylogenetically different groups of orders. These groups are placed in two subclasses:

1. Subclass COCCIDIOMORPHINA Doflein, 1901 emend. Krylov, 1980
   — Order 1. AGAMOCOCCIDIIDA Levine, 1979
   — Order 2. PROTOCOCCIDIIDA Kheisin, 1956
   — Order 3. COCCIDIIDA Labbé, 1889 emend. Krylov, 1980
   — Order 4. ADELEIDA Léger, 1911 emend. Krylov, 1980.
2. Subclass PIROPLASMOMORPHINA Levine, 1961 emend. Krylov, 1980

— Order 1. HAEMOSPORIDIA Danilevsky, 1881 emend. Krylov, 1980

— Order 2. PIROPLASMIDA Wenyon, 1926 emend. Krylov, 1980.

The works of Brugerolle and Mignot (1979), which showed a morphological relationship between *Spiromonas perforans* and SPOROZOA, suggest that SPOROZOA and *Spiromonas* had common ancestors.

More detailed information on the aforementioned subjects can be found in Prof. Krylov's chapter written with Dobrovolsky in the book "Principles of Construction of a Macrosystem of Unicellular Organisms" ("Printsipy Postroeniia Makrosistemy Odnokletochnikh Zhivothykh"), edited by M. V. Krylov and Ia. I. Starobogatov which was published in 1980 as Volume 94 of Trudy Zoologicheskovo Instituta, Akademii Nauk SSSR.

Phylum MICROSPORA

Speaker	Discussant
Dr. Elizabeth Canning (U.K.)	Dr. J. Vávra (Czechoslovakia)

The discussion presented by Dr. CANNING was divided into two parts: (1) Relationship of MICROSPORA with other Protista, and (2) Phylogeny within the MICROSPORA.

(1) Relationship of MICROSPORA with Other Protista

According to Dr. Canning, no obvious relationships appear to exist between MICROSPORA and any of the known groups of protozoa. However, two special structural and biological features of the phylum in question may be significant in speculating on their phylogeny.

MICROSPORA have 70 S ribosomes, precisely comparable with those of prokaryotes. This is surprising in view of their complex structure and development, but may either reflect the level of dependence on their host cells (in common with the absence of mitochondria) or indicate their early branching from the prokaryote/eukaryote stocks.

The second feature of note is the presence, in many genera, of nuclei in diplokaryon arrangement, i.e., a pair of nuclei in close apposition, which divide synchronously. In some genera, both nuclei of the diplokaryon undergo meiosis in preparation for sporogony. Thus, the concept of diploidy, haploidy, and, by inference, sexuality have been established for the phylum. Diplokaryon nuclei and comparable sexual

cycles are otherwise known only in certain groups of fungi, but other biological features common to MICROSPORA and fungi are lacking.

(2) Phylogeny within MICROSPORA

In his new classification of MICROSPORA, Sprague (1977) made use of a range of biological characters in an attempt to reflect evolutionary relationships of the genera. This system is now widely used and is a considerable advance over previous systems.

Dr. Canning limited her comments to the order MICROSPORIDA Balbiani, 1882, which includes all the typical forms. Sprague used the concept of primary dichotomy based on the presence or absence of a pansporoblast membrane around the stages of sporulation (Tuzet et al. 1971). The microsporida belonging to the suborder PANSPOROBLASTINA (Tuzet et al. 1971) sporulate within a pansporoblast membrane and produce spores in groups of 4, 8, 16, 32... n. Those belonging to the suborder APANSPOROBLASTINA (Tuzet et al. 1971) sporulate free in the host cell.

Unfortunately, just as Sprague (1977) published his scheme, the relationship between dimorphism and meiosis in some genera became established and this appears to send the classification of these organisms back to the melting pot.

The dimorphic microsporida have one sporulation sequence, involving meiosis in sporont nuclei (usually in diplokaryon form), giving rise to haploid, uninucleate spores in a pansporoblast. Another sporulation sequence, without meiosis gives rise to diploid (or probably tetraploid) binucleate free spores. The second sequence can be induced by physical stimuli such as temperature or by other biological conditions, e.g., the host tissue in which development takes place, or sex of host. Thus, since pansporoblastic and apansporoblastic development can be induced in the life cycle of one parasite, the separate suborders PANSPOROBLASTINA and APANSPOROBLASTINA are no longer tenable in phylogenetic terms.

In contrast, nuclear cycles may prove useful as an index of affinity. Those microsporida which have a pansporoblastic sporulation sequence involving meiosis (usually with diplokarya) might constitute a natural group, and it is likely that the well known diplokaryotic genus *Nosema* belongs here, although meiosis has not yet been demonstrated in this genus. Other pansporoblastic microsporida, with isolated nuclei throughout their development, must be separated from the dimorphic pansporoblastic forms. An example of the effect of these ideas is the proposal by Canning and Hazard (1981) that the pansporoblastic genus *Pleistophora* be subdivided into three genera to be placed in two fami-

lies: one genus has a meiotic division, the other two have not. To add further confusion, evidence is emerging that the genus *Glugea*, previously considered as apansporoblastic, may indeed be pansporoblastic (Canning, unpublished results).

In conclusion, Dr. Canning suggested that at this time a reappraisal of the classification may be justified at ordinal and familial levels to see whether nuclear cycles can give clearer indications of the phylogenetic relationships of the genera.

The speaker expressed her gratitude to her colleagues, particularly to Drs. E. I. Hazard and J. Vávra (the official Discussant), for discussions from which her ideas were formulated.

(There was little discussion of the Phylum MICROSPORA, beyond the remarks made by Dr. Vávra. Evidently, only relatively few groups of protozoologists are well acquainted with this unique protozoan group.)

Phylum MYXOZOA

Speaker	Discussant
Dr. J. Lom (Czechoslovakia)	Dr. L. Mitchell (U.S.A.)

Dr. LOM remarked briefly about the phylum MYXOZOA, which is unfamiliar to many protozoologists:

1. The phylum is clearly distinct from all the other protozoan groups. *Paramyxidae* can no longer be considered as related to MYXOZOA (see Desportes and Lom 1981);

2. Several characters have been used in support of the relationship between the myxozoans and coelenterates. These included similarities between larval development of narcomedusae and the myxozoans, the existence of parasitic coelenterates, and the close resemblance of morphogenesis of the polar capsules and of the coelenterate nematocysts. Of these, the last feature is the most important; it can hardly represent convergence. It seems that either *Coelenterata* originated from MYXOZOA or might have undergone parallel evolution;

3. The present-day classification of the class MYXOSPOREA is artificial, being based primarily on the spore structure. Shulman's classification (1966), useful in diagnoses, should, however, be retained for practical reasons. Until more natural criteria can be established, only relatively small modifications, including the elimination of the family *Myxosomatidae*, ought to be introduced into this scheme. The natural criteria should be sought among the life-cycle stages (e.g., origin of the

sporoblast), in the fine structure of trophozoites, and in function of cells within a plasmodium.

Since the Discussant failed to supply me with a synopsis of his statement and because there was virtually no discussion on the part of the members of the Panel, nothing further can be reported on MYXOZOA. More time ought to be devoted to this phylum during the next Congress.

Phylum CILIOPHORA

Speaker	Discussant
Dr. Dennis Lynn (Canada)	Prof. J. O. Corliss (U.S.A.)

Many persons, i.e., Professors A. C. Borror (U.S.A.), M. F. Canella (Italy), J. Dragesco (Africa), A. W. Jankowski (U.S.S.R.), and P. de Puytorac (France) were also invited to participate, in the capacity of speakers or discussants, in considering this large and throughly investigated protozoan group. Actually Dr. Lynn's presentation was based to a large extent on the questions raised by Prof. de Puytorac in his abstract printed in the Congress Proceedings. We hoped for a lively exchange, indeed, for some controversy (there are major disagreements among the experts to be found in their publications). For various reasons, the aforementioned workers were unable to join us in Warsaw. The organizers of the Congress and I, as chairman of the Round Table Discussion, are particularly grateful to our young colleague, Dr. Lynn, who with the aid of Prof. Corliss, the Dean of the U.S. "ciliatologists," made an excellent scholarly contribution to our deliberations. (B.M.H.)

According to Dr. LYNN, progress has been made over the past two decades in the systematics of ciliated protozoa. Succeeding the earlier schemes (Corliss 1961, Honigberg et al. 1964), the more recent schemes (de Puytorac et al. 1974, Corliss 1974, 1979, Levine et al. 1980) have incorporated, among other, new ideas on the evolution of nuclear dualism (Raikov 1969), on the relationship between blepharocorythid and entodiniomorphid ciliates (Wolska 1971), and on the similarities in buccal structures of various groups (de Puytorac and Grain 1976).

Small and Lynn (1981) have suggested that the present systems do not recognize monophyletic assemblages if ultrastructure of the cortex is considered. Lynn (1981) has argued that the structural conservatism of biological organization should allow us to place greater weight

on features at the lower levels of biological organization. Hence, Small and Lynn (1981) have relied heavily on ultrastructural features of the somatic cortex to reassess relationships. However, as Patterson and Dryden (1981) have pointed out during the present Congress, structural conservatism is a useful generalization but does not attain the certainty of a law.

In his abstract included in the Proceedings of this Congress, Prof. de Puytorac raised the following important questions, some of which can be addressed from the perspectives that were outlined above:

1. Are somatic cortical features, as used by Gerassimova and Seravin (1976) to define the KINETODESMATOPHORA and POSTCILIODESMATOPHORA, more important than oral cortical features? Oral features are often found to be more variable than the somatic ones irrespective of whether one examines the cortical fibrillar structures (Lynn 1981) or the membrane particle arrays of the cilia (Bardele 1981 a, b). Membership in a group should, therefore, be based first on similarities in somatic ultrastructure features, when present, and then on oral ultrastructure, aspects of morphogenesis, and nuclear characteristics;

2. With respect to oral characteristics, is it of major importance what kind of cytopharyngeal ribbon supports the cytopharynx? Small (1976) has defined two subphyla, the RHABDOPHORA and CYRTOPHORA, on the basis of this feature. Small and Lynn (1981) supported this division and suggested that, at a low level of cortical organization, these structures are reliable indicators of common ancestry;

3. Should SUCTORIA be elevated, as Jankowski (1975) has done, to a rank equivalent to his CILIOSTOMATA and MEMBRANELLOPHORA? One ought to answer in the negative, as the cortical ultrastructures of the swarmers of several suctorian species bear strong resemblances to those of phyllopharyngids and chonotrichs (Lynn 1981);

4. Do KARYOLICTIDA represent a homogeneous assemblage when genera such as *Loxodes* and *Trachelocerca* differ so much in oral structure? If somatic cortical features are truly good indicators of shared common descent, then the karyorelictid ciliates share somatic cortical and nuclear features and are a natural assemblage;

5. Should the colpodid ciliates be elevated to the same rank as VESTBULIFERA? Small and Lynn (1981), on the basis of the uniting features of cortical ultrastructures and stomatogenesis, have separated the colpodids from the vestibuliferans;

6. Should the rhynchodids be united more closely with other phyllopharyngids? On the basis of similarities in the somatic cortical ultrastructure, Small and Lynn (1981) have suggested that this is a reasonable decision to take;

7. Should the nassulids be considered as more closely related to the tetrahymenids, since their oral structures are similar? One is inclined to argue against this because oral structures have a higher probability of being convergent and because the somatic cortical ultrastructures of nassulids place them more closely to the peniculines and microthoracines than to the tetrahymenines (Lynn 1981, Small and Lynn 1981);

8. Should the apostomes be removed from KINETOFRAGMINOPHOREA? Small and Lynn (1981) have argued that this class is not a natural assemblage and should, therefore, be abandoned. Moreover, they related the apostomes to the oligohymenophoreans, because of the resemblance of their somatic cortical ultrastructure;

9. Are toxicysts a phylogenetically important character, since *Protocruzia* apparently has toxicysts? Toxicysts cannot be used as an important diagnostic feature, but they do probably suggest a general common ancestry of larger assemblages of ciliates.

Dr. Lynn has attempted to reply to as many of the questions raised by Prof. de Puytorac as our knowledge at present allows. Answers to questions pertaining to the morphogenesis of budding in suctorians as indicating true diversity, the retention of stichotrichine and sporado-trichine hypotrich groups, or the maintenance of a firm division between pleuronematine and philasterine scuticociliates must await more detailed comparative studies of all these groups.

Prof. de Puytorac has left a most stimulating question to the end. Should the pattern of stomatogenesis always have priority over the pattern of the differentiated oral structures? The speaker would tentatively say yes, although a definite answer requires further, more detailed treatment. Small (1976) has used this approach in establishing the two subphyla; he considered the pattern of differentiation or dedifferentiation of the cytopharynx to be extremely significant. Clearly, this is an area where future discussion is warranted.

The discussant, Prof. CORLISS made the following comments. Dr. Lynn has summarized well most of the principal problems facing ciliatologists today who have an overall interest in the phylogenetics and evolution of the major groups comprising the phylum CILIOPHORA. He has nicely included the controversial questions posed by Prof. de Puytorac, who is sorely missed, in his printed abstract. In fact, based mainly on the recent ideas and conclusions of E. B. Small and Lynn, some still unpublished, Dr. Lynn has offered a new scheme of classification of the ciliates that is, at the very least, stimulatingly provocative.

We have come a long way from the times when the structural diversity and topological distribution of the external ciliature, viewed solely

by light microscopy, were considered sufficient for proposing interrelationships among the suprafamilial taxa of ciliates. The advent of electron microscopy in particular (see history in Corliss 1974) has allowed a revolution not only in taxonomy and classification but also in our ideas concerning affinities and evolutionary lines among these protozoa. New methodologies of data analysis are now available (phenetics, cladistics, etc.) as well as the new technological and cytological approaches: outstanding examples at this Congress would include Dr. Bardele's precise and patient use of the freeze-fracture technique and Dr. Lynn's application of his own "Structural Conservatism Hypothesis." Others have emphasized what the discussant likes to call the "Constellation of Characters Hypothesis;" Drs. Bardele and Lynn also subscribe to this latter approach.

The intriguing case of *Stephanopogon* can be used as a striking example of the value of ultrastructural studies and of treating numerous data by computer analysis. Most of the data Dr. Corliss mentioned — and the half-dozen slides he showed were results of a study now being concluded at the University of Maryland by Ms. Diana Lipscomb.

Stephanopogon has, for a whole century, been recognized and classified as a "relatively simple marine benthic gymnostome ciliate". In a recent book (Corliss 1979), a new order, PRIMOCILIATIDA, was even erected for it. Electron microscopical studies, however, reveal that it not only does not show such major and essentially unique ciliate characters as pellicular alveoli, parasomal sacs, kinetodesmata, and transverse and posticiliary ribbons of microtubules (universally associated with ciliate kinetosomes) but does show such "lower" flagellate features as mitochondrial cristae that are discoidal, a single kind of nucleus with single large central endosome or nucleolus (with "promitotic" type of division), a symmetrogenic mode of fission, a desmose running between adjacent basal bodies, and a subpellicular sheet of microtubules. *Stephanopogon* possesses also some characters found in both ciliate and flagellate groups, as well as several totally unique features of its own. A cladistic analysis of 136 characters, as found (present or absent) in some 34 taxa of flagellated (or ciliated) high-level protozoan groups, reveals that *Stephanopogon* belongs in some supraordinal taxon that also includes both the trypanosomatids (former "lower zooflagellates") and the euglenids (former "green algae"). (Ms. Lipscomb and the discussant will be publishing details soon, elsewhere, including proposal of a unique order for this "ciliate-turned-flagellate.")

DO PROTOZOA CONSTITUTE A "NATURAL" SUBKINGDOM?

Speakers
Prof. J. O. Corliss (U.S.A.)
Prof. G. I. Poljansky (U.S.S.R.)

Discussants
Prof. P. C. C. Garnham (U.K.)
Prof. B. M. Honigberg (U.S.A.)
Prof. W. Michajłow (Poland)
Prof. F. J. R. Taylor (Canada)

The final section of the Round Table Discussion was devoted to the much discussed and controversial question of the subkingdom PROTOZOA. Two of the most eminent students of protozoa were invited to present arguments for and against the premise that the "unicellular" organisms constitute a "natural" subkingdom of the kingdom ANIMALIA. The discussants were also senior scientists who have previously published on this or related subjects.

The following remarks were made by Profs. CORLISS and POLJANSKY, which they summarized in the papers they transmitted to me for inclusion in this report (B.M.H.).

The following text of Prof. CORLISS is given with only minor editorial changes.

We've heard and seen examples throughout papers given at this Congress and especially by the "group-experts" who have participated in this Round-Table Discussion, of w h a t is happening with respect to the systematic arrangements and evolutionary interrelationships among various major protozoan taxa. But no one has yet pinpointed the c a u s e s for what may seem to be "pure chaos" to the "conservative majority" among practicing protozoologists.

The number one point, certainly, is that we're in the middle of a continuing flood of new and exciting data on the protozoa, much of it being of an ultrastructural or biochemical-molecular nature. We have improved methodologies for analysis of numerous comparative data. We are facing up — or need to do so — to the reality, even the practicality, of treating protozoan groups within the larger context of the kingdom PROTISTA, a major taxon of the biotic world containing numerous o t h e r (than protozoan) unicellular eukaryotic forms classifiable into separate classes or phyla.

Every taxonomic protozoologist, although a specialist on his or her own group, should today be aware of the prokaryote-eukaryote story (eukaryogenesis), the serial endosymbiosis theory of Dr. Lynn M a r g u l i s, and the value of recognizing the PROTISTA as separate from the multicellular eukaryotes [kingdoms PLANTAE, MYCETAE (fungi), and ANIMALIA].

Thus, we should view the present ferment or "unrest" as a happy sign of progress, not as undirected chaos.

I wish, as a generalist in protistology, to present briefly an overview of what I have just stated and to answer strongly in the negative the question posed in the title of this final section of our Round Table. I hope to show supportable reasons for rejection of the "PROTOZOA," with a capital "P," as the name for any taxon at phylum, subkingdom, or kingdom levels. To get ahead of my story, there are simply too many intergradations, too many interrelationships of various degrees of closeness of affinity, with other currently (at least) non-protozoan groups of PROTISTA to permit setting up a unique taxonomic barrier between protozoan species of various kinds and members of these other unicellular groups. This works in both (all) directions: the taxa concerned are inextricably commingled, and no longer should we attempt to draw a closed circle around one group, i.e., the former "PROTOZOA." I say this, fully aware of the didactic value of uniting the protozoa taxonomically. Yet it is, as Dr. F. J. R. Taylor has pointed out, the (better) students themselves who are demanding revision of outmoded current classification schemes.

Historically, note the progression from a two- (plants plus animals) to a three-kingdom world (by adding the "PROTISTA," as proposed by Haeckel in 1866). Then note the "regression" to two again, with — mostly by chance, originally — treatment of "algae" and "fungi" (and bacteria) as plants and "protozoa" as ("first") animals.

Using primarily historical groupings (and thus admittedly sometimes quite "unnatural" phylogenetically) of mainly microscopic unicellular organisms above the level of the prokaryotic (super) kingdom Monera (see Corliss, in press in BioSystems), I propose that we recognize the eukaryotic kingdom PROTISTA as being comprised of five major groups. The names I shall use are purposely in the vernacular — they are descriptive of a novel way of packaging some three dozen phyla of protista; and the arrangement should help throw new light on some long-persisting problems, especially with regard to mixed algal-protozoan groupings. It is to be kept in mind, however, that these five groups are not mutually exclusive, that they are not completely "natural" within themselves, and that some are definitely polyphyletic. Thus they serve, in part, to underline my theme that "protists are (simply) protists" and that it is, therefore, unwise and unjustifiable to separate "protozoa" out as a single, monophyletic, unified, definable group, the so-called "PROTOZOA." [Whether the number of "phyla" involved be 10 or 25 (or limited to the 7) of the recent report of the Society (Le-

vine et al. 1980), there is no unique set of supraphylum or subkingdom characteristics that will allow clear-cut taxonomic or evolutionary separation of the protozoa from various other protists.]

Details will appear elsewhere (Corliss, in press, *loc. cit.*), but my five major groupings — containing some 34 phyla, all told — may be listed as follows:

I. The Protozoan Group (with 10 taxa: the "conventional" PROTOZOA but excluding the "phytoflagellates" and certain "slime-mold" groups);

II. The Protozoalgal Group (with 11 taxa: mostly pigmented groups conventionally considered phyla or divisions by phycologists but orders, or lower by protozoologists);

III. The Algal Group (with six taxa: the "usual" algae, including the controversial red and brown algae but excluding forms found above, under II, that would be here in phycological-botanical schemes);

IV. The Protozofungal Group (with four taxa, generally claimed by both protozoologists and mycologists);

V. The Fungal Group (with three taxa, called "lower fungi" by mycologists and to which they wolud add Group IV).

The areas of greatest controversy — with respect to attempts to delimit a single taxon called the "PROTOZOA" — are, of course, within the groupings I've labeled as numbers II and IV. But even with grouping I, the problem of the "multicellular" *Myxozoa* (formerly *Myxospora*) arises. In grouping II, the dinoflagellates have long been mistreated. And the separation of certain forms into separate phyla in groupings II and III — forms which ought to be united under a single phylum (e.g., the "phytomonads" of II and the "chlorophytes" of III) — is unconscionable.

The kingdom PROTISTA itself is difficult to define, at least in a way satisfactory to all. But to attempt to segregate a number of its three dozen phyla into a unique group of "PROTOZOA," (with a capital "P"), is no longer justifiable on the basis of data that have for some time been available to us and that I have attempted to summarize here.

The following remarks were presented by the second speaker, Prof. POLJANSKY. With some editorial changes, they are included here in their entirety.

How should we interpret the question "Do the Protozoa constitute a 'natural' group?" As I understand it, in the conventional sense, i.e., according to Darwin, "a natural group" is an assemblage of organisms related phylogenetically because of their common origin.

Were I asked this question at the end of the 19th or in the early 20th century, my answer would have been simple and unequivocal. At

those times PROTOZOA were regarded as unicellular eukaryotic organisms which gave rise to METAZOA. The origin of the multicellular animals was explained differently by various authors, starting with the gastrea theory of E. Haeckel. However, the most common of the theories proposed through the years was that METAZOA originated from the unicellular eukaryotes, PROTOZOA.

Origins of the protozoan taxa were considered first in the pioneering works of Bütschli (1884, 1885, 1887, 1889). The idea of a monophyletic origin of the PROTOZOA was the basis of all theories on their evolution. Either the naked amebae or the flagellates were considered by different workers as the ancestors of all other eukaryotic groups, including PROTOZOA.

During the last two decades, the originally simple scheme has undergone numerous drastic changes. The great progress in biology, especially in the fields of electron microscopy, molecular biology, and biochemistry, necessitated major revisions of theories previously believed to be unassailable. Among the questions which have arisen as a result of recent developments is whether the PROTOZOA constitute a natural group. This question is associated with the concept of the origin of eukaryotes and with that of their mono- *vs* polyphyletic evolution.

The elucidation of these problems in a manner which would take into account the vast body of information available in published works would necessitate writing an entire book. Such an approach is, of course, beyond the scope of my short presentation. This presentation is intended merely to open a discussion. Because of time limitations, some of the opinions I shall express cannot be illustrated by supporting factual evidence; therefore, my assertions may sound somewhat dogmatic.

The first problem I shall address pertains to the kinds of relationships existing between *Prokaryota* and *Eukaryota*. The question is whether these two main forms of organisms evolved independently of each other or if the latter found their origin in the former.

Both of the aforementioned views find adherents among scientists; I am inclined to support the concept of prokaryote ancestry of the eukaryotes. Indeed, although there exist obvious differences between the two groups of organisms, their most important vital processes are essentially the same. Among the processes shared by the pro- and eukaryotes are: storage, replication, transcription, and translation of hereditary information by DNA and RNA. The common origin of *Pro-* and *Eukaryota* is supported also by their similar batteries of enzymes mediating various metabolic processes. The results of genetic engineering can be used as additional evidence in support of the common origin of the two kinds or organisms, e.g., it is known that some eukaryotic genes

maintain their normal activity when transferred into a prokaryotic cell.

The second problem pertains to the ways in which transition from *Pro-* to *Eukaryota* was accomplished over two billion years ago.

Two main groups of hypotheses have been concerned with this problem.

According to the first theory, the eukaryotic cells originated by symbiosis with prokaryotes, the latter ultimately becoming organelles of the former. The theory of symbiotic origin of the eukaryotes, first formulated by Russian biologists Merezkhovsky (1905) and Famintsyn [1907 (1915)], was subsequently totally forgotten. In recent years it has been revived, mainly due to the publications of Lynn Margulis and her colleagues. Her book, showing the great talent of its author, which appeared in 1970, contains a brilliant and well documented presentation of the symbiosis theory which is considered in terms of modern biology. I shall not go into details of this theory for I am certain that my audience is familiar with the book. I would like, however, to discuss some of the consequences of the concept represented by the symbiosis theory.

The theory allows that eukaryotic cells may have originated from their prokaryotic ancestors on many separate occasions, thus providing conditions for an almost unlimited polyphyly. According to Margulis and her coworkers, the majority of photoautotrophic and heterotrophic flagellates may have originated by symbiogenesis. She placed all the organisms in five kingdoms: Monera (*Prokaryota*), Protists, Fungi, Plants, and Animals. Among the protists, in the broader sense, she recognized 31 phyla that include both the protozoa and algae. MASTIGOPHORA are included in nine of the phyla. Clearly, Margulis does not regard PROTOZOA as a natural group. Indeed, her concept of PROTOZOA appears valid only with regard to a certain level of organization of unicellular eukaryotes belonging to a high but uncertain number of independently evolved groups.

The symbiosis theory is not accepted by all, and its opponents raise serious objections to it. I believe that the transition from *Prokaryota* to *Eukaryota* occurred not by symbiogenesis, but by a progressive increase in the complexity of organization of the prokaryotic cell. Polymerization, as defined by Dogiel (1929, 1954) and his colleagues, i.e., an increase in number of cell components, appears to have played a leading role in this complexity increase.

I shall discuss here only some of the possible objections to the symbiosis theory of eukaryote evolution. As yet, no cases of symbiosis between two prokaryotic cells have been reported. Still, such a relationship is postulated by the proponents of this theory. Most illustrations of transformation of prokaryotic to eukaryotic cells are unconvincing,

e.g., the flagella of MASTIGOPHORA have a complex molecular organization and a typical structure of these organelles (9 + 2 microtubules in the axoneme) is revealed by electron microscopy. This organization, however, has nothing in common with that of either bacterial flagella or with that of spirochetes, which, according to the proponents of the symbiosis theory, have given rise to protozoan flagella. Thus, we are dealing here with superficial analogy rather than with homology.

It is difficult to conceive the kind of symbiont that could give rise to the nucleus of a eukaryotic cell, with its complex organization. However, recent studies of mesokaryotic cells of dinoflagellates (Dodge 1971, Ris and Kubai 1974, Soyer 1972) provide the means for outlining and admittedly only tentative scheme, wherein the prokaryotic nucleoids gave rise first to meso-, and then to eukaryotic nuclei. Of course, I do not mean to imply that modern DINOFLAGELLATA are the ancestors of eukaryotes; actually, I believe this not to be the case.

Certain contemporary workers adhere to the idea of polyphyletic origin of the mastigophorean groups, assuming that the autophototrophs were the primary forms giving rise to the heterotrophs which, therefore, must represent a secondary development (Taylor 1976, 1978). As an all-inclusive generalization, this statement does not appear to be valid. Without a doubt, many groups of autophototrophic MASTIGOPHORA gave origin to the nonpigmented (colorless) ones. There are many examples of this process, e.g., among the euglenids. This does mean, however, that all nonpigmented heterotrophic flagellates had autophototrophic ancestors. Indeed, according to Oparin's theory of the origin of life, certain heterotrophic forms preceded the autophototrophs.

It will be remembered also that metabolism varies greatly among MASTIGOPHORA, different types of metabolism having been observed in closely related organisms. Thus, there are no valid reasons to subdivide the flagellates into several phyla, as was done by Margulis, among others, or even into kingdoms as suggested by Leedale (1974). On the contrary, despite their great diversity, MASTIGOPHORA have many common features of organization, e.g., the structure of flagella, kinetosomes, membranes etc. These common characters enable us to consider the flagellates as one group, with presumably a common origin, which appeared at a certain level of evolution of the eukaryotes. From this viewpoint, the division of MASTIGOPHORA into PHYTOMASTIGOPHOREA and ZOOMASTIGOPHOREA does not appear to be natural.

Among the unsolved questions about the phylogeny of PROTOZOA, there is the relationship between MASTIGOPHORA and SARCODINA. Our present knowledge of the main protozoan groups allows us to recognize the phylogenetic kinships between them. There is insufficient

evidence for the assumption of polyphyletic origins of some of the large assemblages of PROTOZOA. This lack of evidence provides good reasons to regard the foregoing assumption with suspicion.

At present, the correct principles lie in the "Synthetic Theory of Evolution," the modern version of Darwinism, which has been espoused by a number of outstanding scientists (Huxley, Mayr, Dobzansky, and Schmallhausen, among others).

Among the principal lines of evolution of the main protozoan groups, the central position is occupied by MASTIGOPHORA. Their assumption of parasitic existence has resulted in the evolution of the large phylum APICOMPLEXA, among which SPOROZOA are the most important. Equally certain is the relationship between the flagellates and the most advanced protozoan group, CILIOPHORA. The close relationship between MASTIGOPHORA and SARCODINA (above all AMOEBINA) is beyond a doubt. The question of which of the two assemblages originated first has been answered differently by various authors; I do not propose to consider the details of this controversy.

From all the foregoing, it follows that the question of whether the PROTOZOA constitute a "natural" group should be answered in the affirmative.

The PROTOZOA represent a step in the evolution of living organisms characterized by unicellularity and common origin. This statement is not to imply that all PROTOZOA originated from a single "Archiprotozoon" which appeared a very long time ago on our planet. Evolutionary events concerned populations rather than a single organism. It is likely that from the very beginning of life on our planet, evolution was directed toward biocoenoses and to their component populations.

Although we may admit the reality and phylogenetic unity of the subkingdom PROTOZOA, we cannot express a definite opinion as to the evolutionary relationships among all the large protozoan taxa.

CNIDOSPORIDIA may have originated from metazoan ancestors in the course of adaptation to tissue parasitism (Lom, among others). If this hypothesis is valid, the inclusion of the cnidosporidians among PROTOZOA is artificial. It is also unsafe to include MICROSPORIDIA, with their aberrant cell structure, in this subkingdom. It is likely that other smaller protozoan groups also have no phylogenetic relationships with PROTOZOA. None of these facts alter my view that this subkingdom constitutes a natural group which appeared in the course of organic evolution on earth.

Among the discussants of the last section of the Round-Table Discussion, Professor MICHAJŁOW used species of the genera *Euglena* and *Astasia* to show that formal divisions among various protozoan groups,

e.g., between pigmented and nonpigmented flagellates, are always more or less artificial. He also adduced some examples from the group of parasitic euglenids in the hope that information derived from studies of these organisms will aid in the discussion of the larger problem of evolution of PROTOZOA and PROTISTA.

Although, because of the late hour, not much discussion was elicited by the highly interesting contributions of Professors Corliss and Poljansky, it should be mentioned that some of the basic ideas enlarged upon and presented very precisely by Prof. Corliss have been expressed in the 1940's by the late Prof. Harold Kirby (unpublished), by E. N. Kozloff, in his presentation at the Twelfth Meeting of the Society of Protozoologists in 1960 (unfortunately Prof. Kozloff's remarks were not published either in an abstract or in a more complete paper), and by Honigberg (1967). The last author based many of his views on those of Prof. Kirby. Honigberg (1967) ended his paper as follows: "Protozoologists have been concerned with organisms constituting the whole or parts of (some of) the several divisions as considered here [i.e., of RHODOPHYTA, EUGLENOPHYTA, CHLOROPHYTA, PYRROPHYTA, CHRYSOPHYTA, MONADARIA, SARCODINA, CNIDOSPORA (=MICROSPORA and MYXOZOA), SPOROZOA (=APICOMPLEXA), and CILIOPHORA]. Unquestionably they will continue to do so irrespective of the taxonomic treatment of these organisms. Practical considerations such as teaching protozoology on the undergraduate level and aiding the cytologist, the biochemist, the parasitologist, and other nonspecialists, may necessitate, for a time at least, the retention of some of the available and the possible creation of new unified systems of classification of the protozoa. With more information based on light- and electron-microscopic, as well as on biochemical and physiological studies of the various protozoan forms, the kinships within and among the great divisions of living organisms may lead to a more natural arrangement of the groups which are included in these divisions. The probably salutary tendency to elevate the various groups that include protozoa to progressively higher taxonomic levels, clearly apparent in the systems proposed (for example) by Grassé (1952, 1953), Honigberg et al. (1964), Kozloff (unpublished) and Levine et al. (1980) will undoubtedly continue until the generally accepted scheme or schemes may present living things in a manner (similar to that) suggested here." The scheme recommended by Prof. Corliss represents a commendable attempt in this direction.

All the foregoing considerations notwithstanding, the stimulating presentation of Prof. Poljansky, and especially certain parts of it should give us all food for thought.

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Round Table Discussion
Appendix

The Taxonomic Position of *Euglenida parasitica*

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I should like to make some remarks, on the taxonomic and systematic problems of flagellates. I am concerned specifically with euglenids parasitic in copepods, which I have studied since 1955. This group, to which I refer under the designation of "*Euglenida parasitica*", contains now 128 species. It can be assumed that in the future there will be described additional species, the total number being thus doubled or tripled.

Euglenida parasitica is a biologic rather than a systematic grouping. Species, genera, and even families of these euglenids can belong to various taxonomic units within the order *Euglenida*. This indicates that *Euglenida parasitica* is a polyphyletic assemblage.

First of all I would like to join Prof. Poljansky in his opinion, presented in his excellent paper, that there exists a possibility and, indeed, a necessity for determination of species as basic biologic units also among protozoa which undergo exclusively asexual reproduction.

In determination of the *Euglenida parasitica* species the employment of exclusively morphological criteria does not suffice, elthough the significance of these criteria cannot be overlooked. I am certain that many euglenid species described, for example by Skuja or Skovortzova as well as by other authors who based their determinations exclusively on morphologic attributes of flagellated forms swimming in water, will be identified as parasitic organisms after more complete observations of their developmental cycles.

Paper presented at Round Table Discussion on July 11 at VI International Congress of Protozoology, Warsaw, Poland 5–11 July 1981.

Continuous observation of individual cultures is most important in all investigations, because there have been found many euglenids parasitic in copepods which can have several free-living generations in the aquatic environment; yet these euglenids remain obligate parasites of copepods.

I cannot consider here the detailed procedures necessary for species determination; they are described in numerous papers written by me and my coworkers.

The general criteria to be employed in determining parasitic euglenids are as follows: (a) the structure of parasitic and free-living forms (in a life cycle); (b) details of the developmental cycle; (c) typical movement of the parasitic and free-living forms; (d) host specificity based on statistical data; (e) geographic distribution.

Naturally, each of the foregoing categories entails more detailed characteristics. For example, as far as structure is concerned, one can differentiate 15 characteristics of the parasitic forms and an equal number of characteristics limited to the free-living species. Among others, the important characteristics include the type of the mastigont or of paramylon (number, shape, etc. of the paramylon inclusions).

In the course of the developmental cycle, one should consider the method of entry into the host and the site within it (e.g., intestine, body cavity, ovum), the method of leaving the host, the ways of multiplication, and the period of each phase of the developmental cycle, etc. — a total of 25 characteristics. The characteristic movement of the parasitic and free-living forms is difficult to describe; it can be best demonstrated by cinemicrography. One can differentiate several degrees of host-parasite specifity, ranging from very wide to quite narrow. The differences in the degree of specificity depend upon geographical, ecological, and even phenological factors.

Some other criterions of distinguishing the taxonomic units of *Euglenida*, e.g., biochemical and enzymatic properties, which undoubtedly will be taken into consideration in the future. Naturally, the aforementioned characteristics have varying values in differentiating families, genera, and species. For example, the general structure of the mastigont is important in differentiation of suborders and paramylon in that of species.

The parasitic *Dinema* (*Heteronematina*) have different developmental cycles. In this instance parasitism has been the main factor in speciation as well as in micro- and macroevolution. In this suborder we find genera, e.g., *Dinemula*, *Dinemina*, *Paradinemula*, and *Mononema*, containing only parasitic species.

In the suborder *Euglenina* there is the family *Parastasiellidae* consisting of 11 exclusively parasitic species.

What is the placement of various taxa of *Euglenida parasitica* in the new System of Classification proposed by Levine et al. (1980)? This question was the subject of my contribution presented at this Congress (Michajłow 1981). Eighty-three species belonging to partly or entirely parasitic genera can be placed in the suborders *Euglenina, Eutreptiina*, and *Heteronematina*. Forty-five species of the family *Embryocoelidae* have no common characteristics with any of the aforementioned or three other suborders. Therefore, I suggested the creation of a new exclusively parasitic suborder *Embryocolina*, comprising genera with certain morphological characteristics, especially with a mastigont containing an asymmetrically located flagellum which has a characteristic movement as a unit. If this recommendation is accepted we shall be able to assert that (also in this instance) parasitism causing speciation leads toward parasitic megaevolution.

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Symposium B: *In vitro* Cultivation of Parasitic Protozoa

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Chairman: Isabel Cunningham, University of Edinburgh, Edinburgh, Scotland, U. K.
Co-Chairman: Louis S. Diamond, Laboratory of Parasitic Diseases, National Institute of Health, Bethesda, USA
Convenor: Witold Kasprzak, Academy of Medicine, Poznań, Poland

Invited Speakers

C. G. D. Brown	L. D. Hendrics
R. Brun	I. Roitman
L. S. Diamond	W. Trager

The Symposium consisted of a series of papers concerned with *in vitro* cultivation of various protozoan parasites which have a severe impact on the health of man and domestic animals. Since the last Congress of Protozoology in New York in 1977, considerable progress has been made in the cultivation of the various developmental forms found in the vector and in the mammalian host.

Theileria and *Babesia*

C. G. D. Brown traced the history of cultivation of these two closely related species of the order *Piroplasmida*. The marked difference in their life cycle in the vertebrate host is reflected in their course of development of *in vitro* cultivation. *Theileria* sspp. undergo exoery-

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throcytic schizogony in cells of the lymphoid system; *Babesia* multiply exclusively in their intraerythrocytic piroplasm form.

The cultivation of *Theileria* spp. dates from 1945 when Tchernomoretz reported the growth of Koch bodies (schizonts) of *T. annulata* in explants of infected calf spleen placed in drops of calf plasma clots in chick embryo extract and Tyrode's salt solution enriched with various growth-promoting compounds. About twenty years lapsed before the next step of major significance in Theilerial cultivation was recorded. Using *Theileria*-infected cells growing on monolayers of baby hamster kidney cells (B.H.K.), Hulliger et al. (1964) observed the mode of multiplication of the macroschizont in the host cell and demonstrated that parasite and host cell division was interdependent. A few years later successful cultivation of *T. parva* which causes East Coast Fever of cattle in East Africa, was described by Malmquist et al. (1970).

Using the techniques developed for the cultivation of *T. annulata* and *T. parva*, it is now possible to isolate and grow lymphoblastoid cell lines infected with most of the Theilerial species in which schizonts have been described. These include *T. lawrenci* grown in the cells of buffalo and cattle, *T. taurotragi* in eland and bovine lymphoid cells and *T. hirci* in ovine lymphocytes. Cultures of these parasites can be initiated from either schizont-infected lymphoid cells or by *in vitro* infection and transformation of normal lymphocytes by sporozoites of *Theileria* obtained from infected ticks. Establishment of successful cultures in which 90–100% of the lymphoid cells contain theilerial macroschizonts provides an excellent model of parasite-host cell interdependence. These cultures are handled in a manner similar to that used for human lymphoblastoid cell lines isolated from patients suffering from benign or malignant lymphoproliferative disorders.

Methods for the cultivation of *Babesia*, on the other hand, confined to the piroplasm stage within the erythrocyte have only recently been developed. The efforts were undoubtedly stimulated by the reports of the successful cultivation of the erythrocytic stages of human malaria (Trager and Jensen 1976). More recently significant progress has been achieved in the cultivation of *Babesia bovis*. In two distinct, yet related approaches, piroplasms of this important parasite have been grown in bovine erythrocytes in spinner flasks (Erp et al. 1978) and in microaerophilous stationary culture (Levy and Ristic 1980).

Although it is 35 years since the first isolation of *Theileria* in culture, only one stage in its complex life cycle can be grown predictably *in vitro*. It is hoped that the impetus provided by the cultivation of malaria and *Babesia* will produce a system able to support the propagation of the intraerythrocytic piroplasm forms. This perhaps might lead to the production of gametocytes and thus the completion of the verte-

brate cycle *in vitro*. Further concentration on the cultivation of the vector stages of *Babesia* and *Theileria* in arthropod tissue culture would result in the *in vitro* growth of sporozoites useful for possible immunization of mammalian hosts.

Salivarian Trypanosomes

During the past five years there has been remarkable progress in the development of culture systems to support the growth of *Trypanosoma brucei* sspp. and *T. congolense* forms infective to mammalian hosts. Previously only the noninfective procyclic stages could be cultivated with certainty. In his review of these various methods, Dr. Reto Brun also reported in some detail his most significant advances in the cultivation of bloodstream forms of all three Trypanozoon subspecies and their relevance to immunization.

(1) Cultivation of *Trypanosoma brucei* sspp.

(a) Metacyclic Stages

Hitherto when bloodstream forms were added to the commonly used blood-enriched media and incubated at 28°C or 37°C, they transformed into procyclic stages corresponding to those in the tsetse fly midgut and were not infective to mammalian hosts. However, Cunningham and Honigberg (1977) demonstrated that metacyclic forms infective to mice, developed in cultures of procyclics of *T. b. brucei* grown at 28°C in a liquid medium (Cunningham 1977) containing tsetse fly head-salivary gland explants. This culture system, extended and successfully applied to many different stocks of *T. b. brucei* (Cunningham and Taylor 1979) and several stocks of *T. b. gambiense* (Jones et al. 1981), generated metacyclic trypanosomes morphologically (Gardiner et al. 1980 a) and antigenically (Gardiner et al. 1980 b) similar to those transmitted by tsetse flies infected with the same stock. In a series of papers, Nyindo and his co-workers (1978, 1979, 1980) described the cultivation of metacyclic *T. b. brucei* in cultures of bovine cell feeder layers grown in mammalian cell culture media.

(b) Bloodstream Forms

A major achievement in the cultivation of the bloodstream trypanosomes was reported by Hirumi et al. (1977). He devised a system consisting of a feeder layer of bovine fibroblast-like cells grown in

RPMI 1640 with 20% foetal bovine serum for the growth of monomorphic forms of *T. brucei*. A similar technique of growing bloodstream forms in co-cultivation with mammalian cells had been attempted 10 years earlier by Le Page (1967) but gave limited results. Hirumi et al.'s success, however, stimulated further efforts which resulted in the establishment of similar cultures of other *T. brucei* stocks (Hill et al. 1978, Brun et al. 1979, Jenni and Brun 1981).

The following results were obtained by Brun and his collaborators in Basel. Several cell lines and primary cell cultures grown in Eagles MEM supplemented with sera from various animal sources were tested for their ability to maintain the growth of pleomorphic stocks of *T. b. brucei*, *T. b. rhodesiense*, and *T. b. gambiense*. The mountain vole (*Microtus montanus*) embryo fibroblast-like cells (MEF) and rabbit embryo fibroblasts grown in medium with rabbit or human sera were most successful for supporting long term cultivation of blood-stream forms. The cultures could be initiated with either trypanosomes from infected mouse blood or from metacyclic forms from infected *Glossina*. The "cultivated" bloodstream trypanosomes were (a) pleomorphic, (b) infective to mice, and (c) transformed into procyclic forms when added to medium SDM-79 (Brun and Schonenberger 1979) and incubated at 28°C. Of interest was the finding that tsetse fly metacyclic forms transformed rapidly into bloodstream forms in the *in vitro* systems, but without the expression of new variable antigenic types (VATs) during the first 50 h in culture. During this period metacyclic antigens had increased 10–20 fold. At 50 h the trypanosomes were collected, irradiated, and used to imunize mice. These mice were completely protected from infection by tsetse flies' bites containing the homologous *T. brucei* stock.

(2) *Trypanosoma congolense*

Media used by the early workers for the cultivation of *T. congolense* were variations of a liquid medium prepared by combining Ringer's solution with citrated blood (for references, see Tobie 1958). These media and the diphasic blood agar medium of Tobie (1958) were suitable for growing procyclic noninfective trypanosomes transformed from bloodstream forms from mammalian hosts. Trager (1959) observed the growth of procyclic forms in *Glossina* organ cultures using a liquid medium enriched with tsetse pupal extract. The liquid medium of Cunningham (1977) was suitable for the cultivation of procyclics, which had been transformed from bloodstream forms in the medium containing tsetse fly alimentary tract tissues. The culture system of Steiger et al. (1977) consisting of a feeder layer of tsetse fly cells

could support the development of a few epimastigote forms in the procyclic populations.

A cell-free culture system that allows direct adaptation for bloodstream forms into procyclics has been devised in Dr. Brun's laboratory. The liquid medium contains Eagle's MEM with Earle's salts, hemin, and foetal bovine serum. The procyclics reached a maximum concentration of 4–5 $\times$ 10^7/ml.

So far, continuous cultivation of mammalian infective stages of *T. congolense* has not been possible using the methods applied to *T. brucei* sspp. However, the following important advances have provided optimism in this field. The observations of Luckins and Gray (1978) of the close association of *T. congolense* with collagen fibrils in the local reactions at the sites of bites of infected tsetse flies suggested that this material might serve as a useful source of trypanosomes for *in vitro* studies. Indeed, *T. congolense* in cultures prepared from bovine dermal explants taken from local reactions and incubated at 37°C retained their infectivity for up to 21 days (Gray et al. 1979). More satisfactory results were obtained using two unrelated stocks of *T. congolense* established in cultures at 28°C with trypanosomes from proboscides of infected tsetse flies placed beside bovine dermal collagen explants (Gray et al. 1981). The trypanosomes multiplied and formed an adherent layer in the culture flasks. They were subpassaged in medium without dermal explants and retained their infectivity for several months. Infectivity titrations indicated an output of over 10^6 infective organisms per 25 cm^2 culture flask in a 2-day period. The trypanosomes resembled those in the hypopharynx and labrum of a tsetse fly.

(3) *Trypanosoma vivax*

The relatively little attention given to the cultivation of *T. vivax* may be due to the problems of working with this species outside Africa. The first successful attempts to cultivate infective forms were reported by Trager (1959). He found that bloodstream forms from infected sheep added to cultures of organs of *Glossina palpalis* transformed into the various stages found in the vector. Some samples of two culture lines grown for several weeks, and exposed to an elevated temperature of 38°C, developed infections in sheep. During a subsequent visit to the laboratory in Nigeria, Trager (1975) obtained the development and multiplication of epimastigote forms in similar tsetse fly tissue cultures but using a different medium. Older cultures were reported to contain transitional forms almost resembling metacyclic trypanosomes.

During a recent visit to ILRAD in Kenya, Dr. Brun, in collabora-

tion with Dr. S. K. M o l o o, was able to etablish cultures of bloodstream forms of a West African stock of *T. vivax* in feeder layers of goat embryo fibroblast-like cells grown in Eagle's MEM with Earle's salts supplemented with inactivated goat serum. The cultures were maintained for several weeks and were infective to mice. The cultures yielded up to 3 to 8 $\times$ 10^5 organisms/ml and the trypanosomes possessed a surface coat.

These methods for the cultivation of infective trypanosomes offer possibilities of studying processes such as antigenic variation, pleomorphism among bloodstream populations or cell differentiation in the absence of the mammalian host. They might also be useful in development of vaccines and drug testing. It would, however, be desirable if the infective forms could be grown in larger quantities in cell- or tissue-free systems.

Entamoeba and *Trichomonadidae*

Dr. D i a m o n d reviewed the types of media and techniques currently employed in the initiation and establishment of axenic cultures of these protozoa. There are many similarities in the development of axenic culture systems for enteric amoebae and trichomonads. Further refinements and modifications of the available media might still improve the yields of parasites, but satisfactory levels of growth have been obtained for most investigations. Cloning procedures were discussed in their role for providing uniform populations of organisms and as a means of more critically assessing their viability. Relatively few *Entamoeba* or *Trichomonadidae* have been cultivated axenically. The availability of these culture systems would be a useful source of material for studies on biochemistry, physiology and immunology of these organisms. Our knowledge of the metabolism and nutritional requirements of these protozoa awaits the formulation of completely defined media. Furthermore, such media would facilitate investigations of the physical and chemical nature of their biomembranes, a subject of intense interest at present. With few exceptions, the interval between the isolation of these protozoa and their adaptation to axenic cultivation is exceedingly long. Therefore, it is possible that selection and/or mutation could have occurred during this period. There is a need to reduce this period in order to conserve the characteristics of the freshly isolated population. With the exception of *Entamoeba invadens*, a reptilian parasite, the cystic stage of *Entamoeba* does not form spontaneously, and furthermore, encystation cannot be induced in axenic cultures. Thus, this important life cycle stage cannot be studied in other *Entamoeba* under axenic conditions. Knowledge of the biology of cysts is required

for the development of methods to control the transmission of these amoebae, especially the human pathogen, *E. histolytica*. It is important to develop methods to induce encystation under the unique conditions in axenic cultures.

Leishmania

Dr. B. M. Honigberg presented the paper on the cultivation of *Leishmania* submitted by Dr. Larry Hendricks who was unable to attend the Congress. The cultivation methods of the two developmental stages of this parasite were dealt with separately.

(1) Promastigotes

Methods to cultivate promastigote forms morphologically similar to those found in the sandfly have existed for many years. This extracellular, flagellated organism grows optimally at 30°C. The early workers used monophasic or biphasic blood-containing media which have been superceded by the semi-defined or defined liquid media more suitable for assessing the condition and growth of the parasites and for immunological or biochemical investigations.

Commercially available insect media of Grace; Mitsuhashi and Maramorosch, and Schneider (Hendricks et al. 1978, Childs et al. 1978) and the mammalian cell media, e.g., Eagle's MEM and TC 199 (Berens et al. 1976) can generate satisfactory yields of promastigotes of a wide variety of *Leishmania* sspp. These media available commercially, and prepackaged lyophilized with 30% foetal bovine serum in serum vials, have been useful for the diagnosis of both cutaneous and visceral leishmaniasis from both the old and new world. The insect media can support the large scale production of promastigotes for metabolic studies. Several culture systems used for this purpose include the chemostat (Schaefer et al. 1970) and the fermenter (Enders et al. 1977). A typical bulk culture system consisted of promastigotes grown in 9-liter quantities of Schneider's *Drosophila* medium supplemented with 30% foetal bovine serum. Yields of up to 5×10^7 organisms/ml produced 16 ml of packed cells of several strains of *L. braziliensis* and *L. mexicana*. The tsetse fly-trypanosome medium SM (Cunningham 1977) mixed 1 : 1 with RPMI 1640 and enriched with 30% foetal bovine serum has been used routinely for large scale production of promastigotes (5–10 ml of packed cells) of strains of *L. donovani* from East Africa, *L. chagasi* from Central America and cutaneous leishmania from Central and South America and the Middle East.

Chemically defined media are essential for metabolic studies. In 1957, Dr. Trager devised a medium for the *in vitro* cultivation of *L. tarentolae*, a parasite of lizards. Steiger and colleagues (1976, 1977) designed a medium which could support the growth of large populations of *L. donovani* and *L. braziliensis*. Another defined medium described by Berens and his co-workers (1978) was based on their earlier HO-MEM mixture.

(2) Amastigotes

These are round to oval, intracellular organisms which occur in the vertebrate host and can only be grown *in vitro* at host body temperature. Until recently, amastigotes were difficult to collect or cultivate in sufficient quantities for physiological, biochemical or immunological investigations. Limited studies of morphology and O_2 uptake during transformation of amastigotes into promastigotes have been reported by Simpson (1968) and Brun and Krassner (1976).

Mammalian cell cultures can support the growth of amastigote stages with varying degrees of success. The culture system of Walton et al. (1972) could provide enough amastigotes grown in monolayers of VERO (African green monkey kidney) cells for the immunofluorescent antibody test (IFAT), biochemical and metabolic studies. Cells derived from dog sarcoma and hamster peritoneum and infected by promastigotes of *L. tropica, L. mexicana* and *L. donovani* can be used in drug assays (Mattock and Peters 1975). A more authentic system for testing drug activity was described by Berman et al. (1979) in which amastigotes became established and multiplied in human peripheral macrophage cultures: the parasites reside in the macrophages in an infected human host. Promastigotes of *Leishmania* isolated from patients in Schneider's medium for less than a few weeks were infective for human macrophage cells *in vitro*. Using this macrophage-parasite *in vitro* system, it has been shown that patients with a clinical resistance to antimony harbor parasites that are drug resistant *in vitro*. This method was applied to visceral *Leishmania* from Kenya and cutaneous leishmaniasis from Panama.

A technique has been devised for the routine *in vitro* transformation of promastigotes into amastigote-like forms of *L. braziliensis panamensis* and *L. mexicana*. Promastigotes which have entered the log phase of growth in Schneider's medium at 24°C were collected by centrifugation and transferred to medium TC 199 supplemented with 30% foetal bovine serum and incubated at 37°C. Within 48–96 h approximately 95%

of the organisms had rounded up and resembled amastigotes. When these forms were placed in Schneider's medium and incubated at 24°C, they reverted to promastigote forms. The axenically grown amastigotes were slightly larger than those found within the host cell, but their ultrastructure was identical. This source of extracellular amastigotes has been useful for further studies on drug action and determination of biochemical and metabolic properties of the promastigotes, axenically cultivated and host cell-derived amastigotes. Results of preliminary investigations of the infectivity of these stages of *L. b. panamensis* have indicated the axenic and the tissue derived-amastigotes produced cutaneous lesions in African white tailed rats at about the same time (10 days) postinoculation, but much sooner than the promastigotes.

One of the most significant areas to be studied will be the use of the host cell-free amastigotes as antigen in the production of species specific monoclonal antibodies.

Trypanosoma cruzi

This parasite causes Chagas' disease in man. The parasite is widely distributed in Central and South America and extends into the southern United States. The disease in man occurs where dwellings are infested with bugs. Man may contract the disease from bugs which had fed previously on an infected animal.

Dr. Roitman described some of the methods most commonly used to cultivate the different developmental stages of this parasite. The early blood-containing media could support substantial yields of epimastigote and occasional development of trypomastigote forms in cultures incubated at 25–28°C (Taylor and Baker 1968).

Several semi-defined liquid media have been described. In the medium of Citri and Grossowitz (1955) the blood was replaced by haemin, crystalline serum albumin and a series of growth factors. No change in the growth pattern or the yields of *T. cruzi* flagellates occurred during prolonged cultivation in this medium. It was demonstrated by Boné and Parent (1963) that the main function of serum in their medium was as a source of stearic acid, essential for growth of *T. cruzi* epimastigotes. These workers devised a heat-sterilizable medium, composed of peptone and known compounds; it produced maximum populations of 7×10^7 organisms/ml. A macromolecule-free medium, containing in its defined part 3 salts, glucose, haemin, 21 amino acids, 3 lipids and some undefined components obtained by dialysis of liver infusion, was developed by Yoshida (1975) for cultivation of *T. cruzi* at 28°C. The medium supported prolonged cultivation with

growth of the organisms comparable to that obtained in more complex media containing serum.

The complexity of the blood-enriched and semi-defined media had prevented studies on the nutritional requirements of the *in vitro* grown trypanosomes. This was overcome by the development of the defined medium HX-25 of Cross and Manning (1973) originally designed for the cultivation of procyclic forms of *T. brucei*. When *T. cruzi* was cultivated at 28°C in HX-25, the population increased about 10-fold in 10 days, but inocula somewhat larger than those used for *T. brucei* were necessary for good growth. Preliminary data on amino acid utilization indicated threonine uptake and glycine excretion (Cross et al. 1975). Medium HX-25 was also successful in the hands of Anderson and Krassner (1975) who were able to adapt *T. cruzi* orginally grown in a beef heart infusion (BHI) to HX-25 in cultures incubated at 25–26°C. The omission from medium HX-25 of HEPES; KH_2PO_4; $NaHCO_3$; Coenzyme Q_6 and Q_{10} and linoleic acid, and the addition of beta-glycerophosphate Na salt; Na stearate; KCl and $Na_2PO_4 \cdot 12H_2O$ was reported by Azevedo and Roitman (1977) to improve the cultures which attained yields up to 1.6×10^7 organisms/ml incubated at 28°C. The defined medium of Avila et al. (1979) contained bovine liver catalase, horseradish peroxidase, lactoperoxidase and bovine haemoglobin maintained continuous propagation of cultures grown at 27°C. About 95% of the populations consisted of epimastigotes.

The forms which occur in the mammalian host can be grown in association with various cell cultures derived from mammals or man. Of all the cell culture systems tested, human heart cells were considered to be best at supporting good growth of intracellular amastigotes (Brener 1973).

Using two liquid media (F-29 and F-32) Pan (1971) developed a system of cultivation of axenic *T. cruzi* amastigote forms in the absence of host cells. Medium F–29 consisted of TC 199, trypticase, haemin and foetal bovine serum. Medium F-32 was F-29 enriched with chicken plasma. The inocula for serial cultivation in both media contained over 99% epimastigote forms. The proportion of the structural types remained the same during the first 10 passages at 29°C. However, at second passages in medium F-29 at 35.5°C, most of the organisms became amastigotes, and after several more subcultures, they comprised 92% of the population. During the original passage in medium F–32 at 35.5°C over 90% of the parasites were trypomastigotes with a terminal kinetoplast. After further passages, and regardless of incubation temperatures (29.5–35.5°C), over 94% became amastigotes. In both F-29 and F-32 media a seven-fold increase of amastigotes could be achieved after 4 days at 35.5°C.

Malaria

Dr. Trager outlined the most important advances made in the cultivation of *Plasmodium* spp. since the first report of continuous culture of the erythrocytic stage of *Plasmodium falciparum* in human erythrocytes maintained under appropriate conditions (Trager and Jensen 1976). Subsequently the erythrocytic stages of four species of malarial parasites of rhesus monkeys have been placed in continuous culture using similar methods: *P. knowlesi* (Chen et al. 1980, Wicklam et al. 1980) with a 24-hour cycle; *P. fragile* (Chin et al. 1979), a falciparum-like organism; *P. inui* (Nguyen-Dinh et al. 1978) with a 72-hour cycle; and *P. cynomolgi* (Nguyen-Dinh et al. 1980), a vivax-like parasite. A culture of *P. malariae* mixed with *P. falciparum* in human red cells has been reported by Rai Chowdhuri et al. (1979). The second most important human malarial parasite, *P. vivax* has been cultivated by Larrouy et al. (1981) who used methods similar to those applied to *P. falciparum* but with enhanced glucose levels and a change of medium three times daily instead of only once a day. All the culture systems have involved the use of various modifications of the petri dish method or the continuous flow technique originally designed for *P. falciparum*. The most suitable medium seems to be a mixture of RPMI 1640 and human serum which, for some purposes, can be replaced by rabbit serum (Sax and Rieckman 1980) or calf serum with protease peptone (Ifediba and Vandenberg 1980). Most studies are concentrated on *P. falciparum* and several culture lines of this species have been established. There is no indication of loss of infectivity resulting from prolonged cultivation *in vitro*. A morphological change, however, was observed in some culture lines after more than 18 months in continuous culture (Langreth et al. 1979). There was a tendency for the loss of the "knobs," — structures which appear on the membranes of infected erythrocytes when the parasite becomes a trophozoite.

A further advance was the establishment of cloned populations prepared by the dilution method or by microscopic selection of samples with a single organism. These clones seemed to differ with regard to the appearance of knobs on the membrane of the host cell containing trophozoites or late stages (Kilejian 1979). Preliminary studies using clones of a line from Gambia indicated that the knobbed (K^+) clone was infective and pathogenic in *Aotus*, whereas the knobless (K^-) clone produced a barely detectable infection. The K^+ and K^- clones differed also in their *in vitro* response to chloroquine. It is most probable that the culture method, combined with cloning offers good material for studies on the occurrence and nature of drug-resistant strains.

Large scale cultures grown by the method of Trager and Jensen (1980) can produce sufficient yields of parasites for biochemical and immunological studies. Microcultures, on the other hand, have been used to screen for drug sensitivity and to test for growth inhibiting antibodies.

The asexual erythrocytic forms of malarial parasites cannot transmit the infection in nature; when ingested by a mosquito they are killed and digested. However, certain merozoites can develop within erythrocytes into male and female gametocytes. On ingestion by an appropriate mosquito, they form male and female gametes which unite and initiate the sporogenic cycle of development in the vector. Remarkable progress has been made in the *in vitro* production of gametocytes infective to mosquitoes (Trager and Jensen 1978, Jensen 1979) making available for experimental use the sporozoites and the entire sporogenic cycle of *P. falciparum*.

In mammalian malaria, the sporozoites ingested by the mosquito give rise to a single cycle of development in hepatic cells of the liver. The merozoites formed in this pre-erythrocytic cycle seem to be capable only of initiating the erythrocytic cycle. Recently the pre-erythrocytic cycle of rodent malaria, *P. berghei*, has been obtained *in vitro* by inoculation of sporozoites into various types of tissue culture (Strome et al. 1979). The most successful cultures were obtained in a human embryonic lung cell line (Hollingdale et al. 1981) in which large schizonts formed 48 h postinoculation with sporozoites, and the merozoites infective to mice were present at 72 h.

Conclusions

The presentations at this Symposium have demonstrated the continued progress in the cultivation of parasitic protozoa. Methods are now available to cultivate most of the forms found in the mammalian hosts and vectors, but gaps remain to be filled with regard to culture methods for some stages and species of Malaria, Piroplasmida and salivarian trypanosomes. It is likely that before the next Congress this goal will be achieved and thus lead to a better understanding of the parasites.

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PROGRESS IN PROTOZOOLOGY
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Symposium F: Mutualistic (Symbiotic) Relationships

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Co-Chairman: Klaus Heckmann, University of Munster, Munster, FRG
Convenor: Witold Kasprzak, Academy of Medicine, Poznań, Poland.

This section of the program brought together workers whose research interests focused on symbiotic (mutualistic) relationships among protozoa. The program included a Symposium and a Poster Session; thirteen papers were presented, four in the Symposium and eleven in the Poster Session. Topics included algal, bacterial and viral symbionts associated with host protozoa ranging from foraminifera and flagellates to ciliates, both fresh water and marine in origin. For the sake of clarity, this summary is arranged with respect to categories of symbionts discussed.

Algal Symbionts

J. J. Lee and M. E. McEnery found that a number of families of large benthic foraminifera served as hosts for a wide variety of endosymbiotic algae including dinoflagellates, chlorophytes, diatoms and unicellular red algae. As a consequence of protracted and continuous association with these giant protozoa, the outer cell walls of the symbionts, through adaptive mechanisms involving host participation, were repressed within the host and re-formed when released (experimentally) from the host. In most cases, the symbionts, which appeared to be regionally separated from those upon which the protozoans fed, pro-

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vided the host with about 10% of their nutritional needs photosynthetically. Foraminifera containing diatom symbionts were best adapted for photosynthetic activity under conditions where they received 1–10% of the incident light and were capable of effectively recycling nutrients in an otherwise nutrient poor environment. At least one species, *Heterostegina depressa* appeared to be totally dependent on its symbionts to meet its nutritional needs.

D. S. Weis studied the infectious process of algal symbionts of *Paramecium bursaria* and found that infectivity was associated with the ability of the algae to excrete maltose. He observed that, despite a Poisson analysis that indicated a one-hit phenomenon, an average of 1000 algae were required to infect a single ciliate. Because infection frequency was greatly increased when ciliates were exposed to algae intermittantly, rather than in a single dose, he proposed that susceptibility to infection involved an inducible synthesis of receptors located on the surface of the phagosomal membrane and that the inducer was maltose, itself. In support of this hypothesis, the herbicide DCMU which blocks algal maltose release, was found to prevent infection even when present during an initial 30 min period of algal-ciliate contact. The frequency of infection was also reduced when algae were exposed to the lectin concanavalin A. W. Reisser and K. Meier concurred with Weis' finding that the algae excreted maltose and further suggested that the sugar was digested by the ciliates and resulted in an increased growth rate. These workers studied the uptake of algae during infection and found them to be enclosed in perialgal vacuoles with closely attached vacuolar membranes which facilitated nutrient exchange between the partners. It was suggested that host-symbiont specificity may reside in the ability of the host to "recognize" the symbionts. "Unsuitable" algal led to the formation of food vacuoles around them and were presumably digested by the protozoan. Freeze-fracture studies suggested that perialgal and food vacuole membranes differed from each other morphologically.

Bacterial Symbionts

K. Heckmann and R. den Hagen reported on their studies with *omikron*, an infectious gram-negative bacterial endosymbiont found in the fresh water ciliate *Euplotes aediculatus*. Destruction of the symbionts by treatment with penicillin caused the ciliates to die unless reinfected with *omikron*. All *Euplotes* with a 9-type 1 fronto-ventral cirrus pattern were found to contain and be dependent upon symbionts for cell division. *E. crenosus* and *E. palustris*, both characterized by 10 fronto-ventral cirri and *E. musicola* (9-type 2 pattern) did not contain symbionts. Over 40 stocks representing 7 species were examined.

Only those with 9-type 1 fronto-ventral cirri contained symbionts. J. A. Kloetzel exposed *omikron*-containing *Euplotes aediculatus* to gamma irradiation in an attempt to produce amicronucleate strains and found the greatest effect to be on the symbionts. Animals exposed to 40 Krad usually died by day 17 whereas cells exposed to 23 Krad survived and divided after a lag of several days. Survival was attributed to the re-establishment of normal-appearing symbiont populations, a result consistent with Heckmann's finding that *omikron* was essential for the growth and division of *E. aediculatus*. A. T. Soldo studied bacterial-like symbionts, termed xenosomes, of the marine ciliate *Parauronema acutum*. Xenosomes, found in 12 strains of the protozoan, were gram-negative, contained RNA, DNA and protein, divided in synchronism with the host and were susceptible to the action of a number of antibiotics. Unlike *omikron* particles, xenosomes were not essential for the growth of the protozoan. Rather, they appeared to be dependent upon the protozoans for growth. Xenosomes were found to infect homologous as well as heterologous *Parauronema* cells. Some strains were capable of killing certain species of the genus *Uronema*. The structure and size of chromosomal xenosomal DNA was unusual for a bacterium. There were 8 copies of a circularly permuted DNA molecule of MW $= 0.34 \times 10^9$; most free-living bacteria contain one (or at most two) copies of the genome and are much larger with respect to molecular weight. H-D. Gortz described a symbiotic bacterium, *Holospora elegans* that multiplied exclusively in the micronucleus of *Paramecium caudatum*. After being taken up in the food vacuole together with prey organisms, the infectious form of the symbiont underwent a series of morphological changes and was carried in a "transport vessel" to the micronucleus. The tip of the symbiont, which may be seen with thin fibrils projecting from it, entered the micronucleus and established the infection. In other work with *P. caudatum*, J. Dieckmann described gram-negative bacterium-like symbionts, 4–10 μm long and 1–2.5 μm wide, which were present in the cytoplasm. The symbionts contained refractile inclusion bodies that differed in appearance from the R-bodies of *kappa* and were motile, propelled by flagella, when released from the ciliate. The symbionts were capable of infecting syngens 1, 3, 12 and 13 of *P. caudatum* but not other ciliate species. Unless the paramecia were grown at 2–3 fissions per day the symbionts multiplied and killed the host. A. I. Radchenko reported on the presence of gram-negative bacterial symbionts in the nucleus of the euglenoid flagellate *Peranema trichophorum*. The symbionts were 1–2 μm long and 0–3 μm wide. The cell wall was 20–30 nm thick. Nucleoids resembling DNA of bacteria were observed; ribosomes, 17 nm in diameter, were also present. The symbionts were non-motile and did not

possess flagella. Bacterial symbionts occurring in dinoflagellates were studied by autoradiographic techniques and by fluorescent microscopy by S. Franca and E. S. Silva. These workers concluded that the symbionts gained entrance into the dinoflagellates early in its life cycle.

Viral Symbionts

J. Teras and L. Kesa studied protozoan-viral interactions using *Tetrahymena pyriformis,* myxovirus and picornaviruses. Previous studies showed that whereas *Tetrahymena* served as host for picorna-viruses, myxovirus was rapidly inactivated by the protozoan. However, previous exposure of *Tetrahymena* to myxovirus rendered the protozoan incapable of harboring picornavirus, thus establishing for the first time, viral interference in a protozoan-virus system. In studies of the interaction of *Giardia duodenalis* and picornaviruses *in vitro*, G. Takhonia, J. Teras and L. Kesa found that the picornaviruses were not only taken up by the protozoans but were capable of exerting a cytopathogenic effect on new-born white mice even after several passages of the infected protozoan in virus-free medium. Further, the virus could be re-isolated from the protozoans even after several subcultures in virus-free medium. These results suggested that the cytopathic effects of the virus remained unchanged as a consequence of protracted residence in the protozoan host. S. Perez-Prieto, M. A. Jareno and A. Garcia Cancedo studied the uptake of vaccinia virus in ciliates. In experiments carried out at 25°C, extracellular viruses disappeared progressively, although low levels of viral activity remained for some time. In similar experiments with *Onycchodromus acuminatus,* viral liters decreased to non-detectable levels. Future experiments were planned with *T. pyriformis* strains adapted to 37°C.

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Symposium A

The Molecular Diversity and Evolutionary Antiquity of the *Tetrahymena pyriformis* Species Complex

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(1) Evolutionary Events Can be Reconstructed from the Comparative Anatomy of Molecules

In the 19th Century a major tool for reconstructing evolutionary events was comparative morphology, and this tool continues to be an essential instrument of the evolutionist. Improved technologies, however, enable us to compare structures of ever smaller dimensions; comparative ultra-structural analysis is becoming a powerful means of probing protozoan phylogenies. The ultimate comparative anatomy is, however, the comparative anatomy of molecules. We are now able to detect sequence differences in the components (nucleotides or amino acids) of the major informational macromolecules, and we are beginning to acquire the ability to compare secondary and tertiary configurations also. A major advantage of comparative biochemistry is that it reveals relationships much deeper into the past, even beyond the records of the rocks into the remote times of life's beginnings.

A recent splendid example of the uses of comparative molecular biology is provided by the work of Carl Woese and his colleagues (Woese 1981) on the nucleic components of the ribosomal apparatus.

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Basing their analysis on the primary structure of the ribosomal ribonucleic acids, these workers have stretched our understanding of evolutionary events back almost to the beginning of life on earth, to the origin of the ancestor of modern molecular information-processing systems. All life forms use essentially the same nitrogenous bases in their DNA and RNA, the same amino acids in their proteins, the same ribosomal apparatus and the same genetic code to translate molecular information. No stronger argument is available for the unity of origin and universality of design of all forms of life than is found in the commonalities of the ribosomal apparatus.

But the ribosome, although conservative, is not invariant. Subtle differences can be found among the ribosomal molecules of different organisms. Organisms of similar appearance, showing close relationships by more conventional criteria and through the fossil record, also show few differences in their RNA sequences. Organisms judged to be of more remote common ancestry have more sequence differences. If one assumes that sequence differences are a measure of evolutionary distance, even beyond the limits of conventional taxonomic criteria and paleontological records, one can reconstruct the previously hidden first half of the history of life.

This analysis stretches back to the "progenote", this first (or most) successful self-perpetuating information-processing machine, that managed to become the granddaddy of us all. The time of origin of the ribosome, and its bearer, is still uncertain, but geochemists are progressively moving back their estimate of the time when life began to leave chemical traces in the earth and the atmosphere. Some now estimate that life was present in quantity some 3 1/2 or 4 billions of years ago, within 1/2–1 billion years after the origin of the earth. The ribosome is arguably as ancient as that. The ribosomal analysis also shows an unexpected evolutionary cleavage among the organisms previously lumped together as bacteria, or prokaryotes. The Woese group shows two distinctive phylogenies — that of the common eubacteria and the much rarer archaebacteria, which may have been common in earlier times, but which are now driven into relict habitats, in salt marshes and ocean depths. The ribosomal apparatus of eukaryotic cells apparently came from the same ancient source, and its sequences also drifted slowly through the long eras before the appearance of modern eukaryotes.

This molecular reconstruction of early evolutionary history, though as yet skimpy in detail, reveals the power of the technique. It also leads to new perspectives on the origins of the eukaryotes, and renders obsolete many of the perennial systematic arguments concerning the eukaryotic protists. We cannot yet answer all the questions concern-

ing eukaryotic beginnings and protistan relationships, but the means are at hand for definitive solutions and they will soon be ours.

The first modern eukaryote probably appeared on the evolutionary scene something like 1.8–2.0 billions of years ago, about half the time since the appearance of the progenote. It likely appeared as the result of a single improbable collusion — a genetic conspiracy involving two or more evolutionary lineages that had been separated for some 2 billions of years. The saltation (or punctuation) created in some as yet uncertain way a "community" organization with vastly improved capabilities. This organization involved at least three major improvements: a plasma membrane capable of phagocytosis, pinocytosis, excitability and enhanced sensitivity; a microtubular system capable of forming the 9 + 2 external motility organelles, and the corresponding centrioles and associated elements responsible for moving internal structures; the nucleosomic apparatus, consisting of the 5 classes of basic proteins called histones, which make chromosomes possible and through them the storage and manipulation of orders of magnitude more nucleic information than could be managed by more primitive organisms.

The important thing for present considerations is that the new eukaryote inventions, like the earlier ribosome, were quickly brought to a state of efficiency incapable of improvement without radical redesign. The 9 + 2 structure of cilia and flagella does not vary significantly between *Chlamydomonas* and *Homo*. The excitable membrane of *Paramecium* has properties remarkably similar to those in the nervous system of the squid. The nucleosomes of *Tetrahymena* are scarcely distinguishable from those of *Drosophila* or maize.

These fundamental eukaryotic structures provide, therefore, conservative molecules whose variations can be used to measure large evolutionary distances. Not all molecules are equally useful in different kinds of evolutionary enquiry. Variations between certain histone molecules are essentially useless in studying vertebrate relations, for example; the vertebrates are of far too recent origin to have accumulated a statistically useful number of histone differences. On the other hand, evolutionarily labile molecules such as esterases may be useful in discriminating among insect subspecies, but may be of no value in examining more ancient evolutionary separations; they are so variable as to supply only meaningless noise when the organisms being compared are of more distant relationships.

I apologize (insincerely) for taking so much time to display my prejudices concerning the principles of molecular evolution, and also for not documenting more fully the evidences for them. Time does not permit a full explanation, however, and a summary is necessary for

background against which to examine the molecular variety of the *Tetrahymena pyriformis* complex.

(2) *Tetrahymena* Arose Very Early after the Eukaryotic Saltation

In principle the techniques of molecular biology are capable of unrevelling the tangled history of life in whatever detail may be desired. In practice their application is constrained. Some of the techniques require high technology, equipment and expertise not available to everyone. An even more severe restriction is the need, in most cases, to have at least semi-domesticated organisms for study. The organisms need to be capable of growth under controlled conditions which permit the introduction of appropriate labels into their molecules. The happenstance of *Tetrahymena's* domesticity, first demonstrated by Lwoff (1923) over half a century ago, is responsible for the abundance of information about its molecular characteristics. *Tetrahymena* has been a favorite object of biochemical analysis in dozens of laboratories for dozens of years. Unfortunately, this work has not been systematic; it has not always been applied to properly identified strains, and it has not always been viewed in appropriate evolutionary perspective. The useful information is fragmentary.

I will briefly summarize here some of the studies on *Tetrahymena* that place it in the context of the broad evolutionary tapestry I displayed earlier. I wish I could show you the 16S ribosomal RNA from *Tetrahymena*, but this is not yet available. Perhaps the next best thing is the 5S ribosomal RNA recently reported by Luehrsen et al. (1980). The *Tetrahymena* 5S RNA is without question a true eukaryotic 5S RNA, but it has some features which are distinctive. It contains, for example, a sequence CGAAC beginning at position 40 that had previously been found in all eubacterial 5S molecules but in no eukaryotic 5S molecules. This molecule from *Tetrahymena* is, with the exception of that from yeast, the most atypical eukaryotic molecule that has been examined. The force of this statement is weakened by the realization that relatively few eukaryotic 5S RNA molecules have been sequenced. *Tetrahymena thermophila* is the only *Tetrahymena*, the only ciliate, the only protozoan according to a restrictive definition, for which we have 5S RNA data. Nevertheless, the data indicate that *Tetrahymena* branched off the eukaryotic stem early; precisely how early is still in question.

A second reputedly conservative molecule for which we have some *Tetrahymena* data is histone H4. On superficial analysis, this chromosomal constituent seems to be very much like all other H4 molecules

in eukaryotes. It is about the same size as the others, and has about the same amino acid composition. The *Tetrahymena* molecule seemed to confirm the reputation of H4 as the most conservative protein molecule known. Thus far, three mammalian H4 molecules have been sequenced; no differences were found among the pig, the calf and the rat. The sea urchin differs from the mammals in one amino acid substitution; the pea plant differs in two amino acid replacements. But when Glover and Gorovsky (1979) began to sequence the *Tetrahymena* H4 molecule, the conservatism was shattered. The molecule has been only partially sequenced, but it already differs from mammalian H4 in at least 15 respects, including a deletion, an insertion, and many changes of amino-acid type. *Tetrahymena* has the most aberrant H4 molecule thus far sequenced.

Once again, this one item of information does not tell us very much about the relations among the ciliates or between them and other protists. It is consistent with a very early separation of the ciliates from the main root of the eukaryotes.

Another protein very important in evolutionary considerations is cytochrome c. The evolutionary history of this protein is fascinating, because the protein appears in both eukaryotes and in eubacteria. Cytochrome c is a mitochondrial enzyme and is widely believed to have come into the eukaryotes along with the captured mitochondrial genetic system at the time the modern eukaryotes appeared. In any case, the ubiquity of the protein and the substantial comparative information concerning it make it a useful phylogenetic marker. G. E. Tarr (cited as personal communication in Ragan and Chapman 1978) has determined the amino acid sequence of cytochrome c from *Tetrahymena*, and has concluded that it is the most atypical eukaryotic cytochrome c thus far sequenced. In this case the evidence for a unique phylogenetic position is somewhat stronger than in the case of 5S RNA and histone H4, because more comparative data are available. At least *Crithidia*, *Euglena* and *Physarum* seem to be of later phylogenetic origin.

One final conservative molecule, in this case a conservative protein of the plasma membrane, supports the evidence of the molecules previously considered. Nozawa and Nagao (1981) have reported at these meetings the primary structure of a *Tetrahymena* calmodulin. They review the evidence for homologies of the molecules compared and show that the *Tetrahymena* protein is the most distinctive of the calmodulins thus far studied.

Inferences based on any one of these molecules are of uncertain reliability, particularly because of the lack of an adequate comparative base. In conjunction, however, the four molecules allow a strong supposition that *Tetrahymena*, and coincidentally the ciliates generally, were

among the earliest of the major eukaryotic protists, at least the earliest represented by extant forms. Perhaps the discovery of nuclear compounding provided the first effective means of increasing greatly the size and motility of the eukaryotic organisms. And perhaps these new capabilities permitted an important early radiation. That radiation could conceivably have occurred as early as 1.5 to 1.8 billion years ago, but the time cannot be fixed until more satisfactory data are available for several well-calibrated presumably chronometric molecules.

(3) *Tetrahymena pyriformis* is a Complex of Species Remarkably Similar in Certain Features

Following Sonneborn's (1937) discovery that *Paramecium aurelia* is a cluster of sibling species, many named ciliate species have been shown to be species complexes. The first systematic study of the population structure of *Tetrahymena pyriformis* was carried out by Gruchy (1955), who found eight genetically isolated species in collections from North America. Elliott and his collaborators continued

Table 1
Some mating groups in the *T. pyriformis* complex

Species Name		*T. thermophila* I	II	III	IV	V	VI	VII	*T. pigmentosa* I	II	III	*T. canadensis* I	II	III	IV	V
T. thermophila	I	−	+	+	+	+	+	+	−	−	−	−	−	−	−	−
(mating group 1)	II		−	+	+	+	+	+	−	−	−	−	−	−	−	−
	III			−	+	+	+	+	−	−	−	−	−	−	−	−
	IV				−	+	+	+	−	−	−	−	−	−	−	−
	V					−	+	+	−	−	−	−	−	−	−	−
	VI						−	+	−	−	−	−	−	−	−	−
	VII							−	−	−	−	−	−	−	−	−
T. pigmentosa	I								−	+	+	−	−	−	−	−
(mating groups 6, 8)	II									−	+	−	−	−	−	−
	III										−	−	−	−	−	−
T. canadensis	I											−	+	+	+	+
(mating group 7)	II												−	+	+	+
	III													−	+	+
	IV														−	+
	V															−

these explorations, making collections in Central America, the Pacific Islands, Australia and Europe (see Elliott 1973). The general situation is illustrated in Table 1. Each species, once referred to as a "variety"

Table 2

Mating species of *Tetrahymena pyriformis* complex

Species Name	Syngen (Mating Group) Number	Collector	Number of Mating Types	Mode of Mating Type Inheritance
T. thermophila	1	Elliott and Hays (1953)	7	karyonidal
T. americanis	2	Gruchy (1955)	9	synclonal
T. borealis	3	Gruchy (1955)	7	karyonidal
T. hegewischi	5	Gruchy (1955), Nyberg (1981)	8	unknown
T. canadensis	7	Gruchy (1955)	5	karyonidal
T. pigmentosa	6,8	Gruchy (1955)	3	synclonal
T. tropicalis	9	Elliott and Hays (1955)	5	unknown
T. hyperangularis	10	Elliott et al. (1962)	4	synclonal
T. australis	11	Elliott et al. (1966)	3	unknown
T. capricornis	12	Elliott et al. (1966)	4	unknown
T. sonneborni	13	Nyberg (1981)	3	unknown
T. nipissingi	14	Nyberg (1981)	3	unknown
T. malaccensis	16	Simon (1981, unpublished)	6	karyonidal

or "syngen" but now given a Latin binomial (Nanney and McCoy 1976), contains two or more different mating types which mate in all heterologous combinations within a species. The 13 currently recognized breeding species (Table 2) include three recently named by Nyberg (1981) and one discovered by Simon (personal communication) in Malaysia.

Not all strains conforming to the type *T. pyriformis* (see Corliss 1952, 1979) are represented in the list of breeding species. A considerable fraction isolated from nature self in unmixed cultures and cannot therefore be assigned to species by mating reactions. An even larger number of isolates are amicronucleate, and generally these amicronucleate strains cannot be induced to interact with mating strains. Relatively little attention has been paid to the selfing and amicronucleate strains, but several amicronucleates have found their way into research laboratories. Although they cannot be classified by mating reactions, amicronucleates can be readily discriminated by molecular analysis (Borden et al. 1973 a) and four groups have been assigned Latin binomials (Nanney and McCoy 1976). The amicronucleate "species" seem to have few distinctive characteristics in common. None has been convincingly associated with any particular micronucleate species.

The genus *Tetrahymena* contains at least 8 "morphological" species in addition to the *T. pyriformis* group (Corliss 1979). Some of these are certainly species complexes also, but in the absence of adequate breeding and systematic biochemical analysis, the extent of species crypticity in them is uncertain. Our present understanding of the complexity of the genus is summarized in Table 3.

The genetic isolation of the cryptic species of *T. pyriformis* has provoked several efforts to find some useful biological correlates of their evolutionary disjunction. Generally speaking, studies of supramolecular characteristics have not been very helpful in this respect. I will not discuss all these inconclusive studies, but will summarize briefly some representative ones.

First, let us consider size. The size of a ciliate varies by a factor of two in each replication cycle, and it is also sensitive to growth conditions. To assess size accurately, one must control the cultural conditions and examine cells at a fixed time in the cell cycle. One measure of size (surface area) is provided by an assessment of the number of ciliary units (basal bodies) on the cell surface. It can be converted to a measure of mass and length, but can also be left as a surface measure. In a comparative study (Nanney et al. 1978) the number of basal bodies in the posterior cell at division was found to vary from a low of 234 in the amicronucleate *T. furgasoni* to a high of 481 in *T. capricornis*. Assuming equivalent sizes for cortical units in the species, the surfaces vary by a maximum factor of about two. Again making plausible but

Table 3

The genus *Tetrahymena*. The taxa on the left are defined on conventional morphological criteria; those on the right employ genetic and biochemical distinctions

Pyriformis complex	Amicronucleate species (Phenosets)
	(a) *T. pyriformis* (*sensu stricto*) (A)
1. *T. pyriformis*	(b) *T. elliotti* (B)
	(c) *T. furgasoni* (C)
2. *T. setosa*	(d) *T. lwoffi* (E)
3. *T. chironomi*	
Rostrata complex	⋮
	n
1. *T. rostrata*	Mating species — Micronucleate (Syngens)
2. *T. limacis*	(a) *T. thermophila* (1)
	(b) *T. americanis* (2)
3. *T. corlissi*	(c) *T. borealis* (3)
	(d) *T. hegewischi* (5)
Patula complex	(e) *T. canadensis* (7)
	(f) *T. pigmentosa* (6, 8)
1. *T. patula*	(g) *T. tropicalis* (9)
	(h) *T. hyperangularis* (10)
2. *T. vorax*	(i) *T. australis* (11)
	(j) *T. capricornis* (12)
3. *T. paravorax*	(k) *T. sonneborni* 13)
	(l) *T. nipissingi* (14)
	(m) *T. malaccensis* (16)
	⋮
	n

non-rigorous assumptions, the mass of the smallest species is about 1/4 that of the largest, its length is about 68%. The mean sizes (Table 4) of the species form a continuous and highly constrained series. Size is not a useful diagnostic characteristic.

A second characteristic that has received considerable attention is the *Tetrahymena* cytoarchitecture (Nanney et al. 1980). For illustrative purposes, I draw attention to the location of the contractile vacuole pores (CVP), approximately 1/4 of the circumference of the cell to the right of the stomatogenic meridian. This position does vary significantly among the species. In *T. thermophila* the mean position is 21.3% of the circumference; in *T. hyperangularis* it is 29.8%. All the other means (Table 5) are intermediate, and tightly clustered at about 25%. The CVP position is a representative value indicative of the form of the cell, its basic geometrical organization. It, like size, varies continuously within very narrow limits, and is not useful in discriminating species.

Table 4

Measures of the size of presumptive opisthe Tetrahymenas from fast exponential growth. Only the basal bodies were enumerated; the volume and length estimates are based on geometrical extrapolations

Species	Surface Mean Basal Bodies	Volume Arbitrary Units	Length Arbitrary Units
Amicronucleate species			
T. pyriformis (A)	362	131	19
T. elliotti (B)	317	100	18
T. furgasoni (C)	234*	55	15
T. lwoffi (E)	282	80	17
Micronucleate species			
T. thermophila (1)	369	136	19
T. americanis (2)	434	188	21
T. borealis (3)	400	160	20
T. hegewischi (5)	352	124	19
T. canadensis (7)	447	200	21
T. pigmentosa (6)	440	194	21
T. pigmentosa (8)	418	175	20
T. tropicalis (9)	381	145	20
T. hyperangularis (10)	365	133	19
T. australis (11)	447	200	21
T. capricornis (12)	481*	231	22
T. sonneborni (13)	NA	—	—
T. nipissingi (14)	391	153	20

Nanney et al. (1978).
* indicate extreme values.

I will document the constrained variability of only one additional gross cellular characteristic, that provided by a recent study of Doerder et al. (1981) on the DNA content of the macronucleus (Table 6). This study was not so complete with respect to the species of the *T. pyriformis* complex; Doerder's group examined only three amicronucleate species and five mating species. But they also examined strains of four other species within the genus. With respect to the variation within the *T. pyriformis* complex, we note about the same kind and degree of quantitative variation observed with basal body numbers. The smallest species is again *T. furgasoni*, but the largest species measured was one of the *T. pigmentosa* subspecies. The smallest species has about 1/3–1/4 the DNA content of the largest species.

Even this variability, though not large, may nevertheless be exaggerated. The macronucleus divides "amitotically", and the products are only approximately equal (Cleffmann 1974). The dispersion of DNA content within the clone becomes large, as can be seen from the coeffi-

Table 5

The locations of the contractile vacuole pores on the cell surface, measured as the fraction of the circumference of the cell from the oral kinety

Species	Mean Location of CVP's % of Cell Circumference
Amicronucleate species	
T. pyriformis (A)	22.5
T. elliotti (B)	23.5
T. furgasoni (C)	24.5
T. lwoffi (E)	24.8
Micronucleate species	
T. thermophila (1)	21.3*
T. americanis (2)	24.9
T. borealis (3)	22.0
T. hegewischi (5)	24.5
T. canadensis (7)	22.5
T. pigmentosa (6)	23.9
T. pigmentosa (8)	23.8
T. tropicalis (9)	24.5
T. hyperangularis (10)	29.8*
T. australis (11)	24.3
T. capricornis (12)	24.8
T. sonneborni (13)	24.6
T. nipissingi (14)	29.7

Nanney et al. (1980).
* indicate extreme values.

cients of variation. Moreover, the DNA content is slightly heritable in vegetative growth; a cell with low content tends to produce daughters with low content. Rectification occurs episodically, by means of an added round of DNA synthesis or an additional cell division without an intervening S phase. Nevertheless, the subline variability in DNA content is large enough to cast doubt on the significance of the differences in the means. DNA content is not a useful diagnostic character.

The measurements of DNA content in other species show that the entire genus is similar in scale, though differences in other characteristics are well established. The high coefficients of variation in the other species indicate that DNA content is regulated in much the same imprecise way as in the *T. pyriformis* species cluster.

Generally, attempts to distinguish among the species of this cluster using gross organismic characters have had limited success. Either the species are tightly clustered according to some numerical measure, or they show no variation at all. Charles Ray Jr. (1956), for example, studied the chromosomes in the micronuclei of several species (*T. thermo-*

Table 6

Estimates of the mean DNA contents of *Tetrahymena* strains in picograms DNA per G2 macronucleus

Species	Mean	Coefficient of Variation (%)
T. pyriformis complex		
Amicronucleate species		
T. pyriformis (A)	30	25
T. elliotti (B)	15	17
T. furgasoni (C)	13*	16
Micronucleate species		
T. thermophila (1)	20	20
T. americanis (2)	30	20
T. canadensis (7)	20	20
T. pigmentosa (6)	25	25
T. pigmentosa (8)	36*	20
T. hyperangularis (10)	35	20
T. rostrata complex		
T. rostrata	40	25
T. corlissi	50	25
T. patula complex		
T. patula	40	12
T. vorax	30	30

Doerder et al. (1981).
* indicate extreme values.

phila, *T. americanis*, *T. hegewischi*, *T. pigmentosa* and *T. tropicalis*) and concluded that the five pairs of chromosomes observed in each were indistinguishable. Similarly, Elliott (1973) notes the "remarkable homogeneity" of the species he surveyed for nutritional requirements. Although occasional deviant strains have been reported, and mutations have been induced, the standard nutritional pattern of the 10 species studied is to require 11 amino acids, 7 B vitamins, a purine and a pyrimidine.

In all the gross organismic characteristics — size, shape, karyotype, biosynthetic processes — the *T. pyriformis* complex is monolithic. It is against this organismic homogeneity that we have to consider the question of molecular diversity.

(4) The *T. pyriformis* Complex is Enormously Diversified in its Molecular Characteristics

The impression of phenotypic invariance, or at least of tightly constrained phenetic variability, is sharply in conflict with essentially every

investigation of the molecular or genetic diversity of the *T. pyriformis* complex. Once again I must select examples rather than catalog exhaustively, and will choose preferentially studies of supposedly conservative molecules.

The first molecular characteristic is chosen in part for historical reasons. Sueoka's (1961) study of the base composition of several strains of *Tetrahymena* provided the first indication of vast genetic distances among these species. All organismic DNA's contain of course the same nitrogenous bases: guanine and cytosine, thymine and adenine. And they contain these in certain fixed proportions, e.g., the molar amounts of thymine and adenine are equal. The relative amounts of G and C relative to the amounts of A + T are, however, permitted to vary over a considerable range in different organisms. Within a species the average base composition is a highly conservative characteristic. Closely related species usually have identical base compositions. Among all the vertebrates, the base composition varies only about 4%, from 40% G + C to 44%.

Yet, all the workers who have studied several *Tetrahymena* strains have reported readily detected differences in base composition. The 12 strains studied by Sueoka (Table 7) had G + C values ranging from

Table 7

Percent C+G/total bases in total DNA from different *Tetrahymena* strains

Species	Investigators			
	Sueoka	Suyama	Allen, Gibson	Flavell, Jones
T. pyriformis complex				
Amicronucleate species				
GL (species unknown)	30	28		26
E (species unknown)	31			
ST (species unknown)	33			
W (species unknown)				33
Micronucleate species				
T. thermophila (1)	25	25	24, 25, 26	25
T. americanis (2)	25			
T. borealis (3)	27			
T. hegewischi (5)	25			
T. canadensis (7)	27		25, 26	
T. pigmentosa (6)	25	25		
T. pigmentosa (8)	25			
T. tropicalis (9)	28	30	28	
T. hyperangularis (10)			31	
T. australis (11)			30	
T. capricornis (12)			25	
T. rostrata	23			

23–33%. Later workers (Suyama 1966, Allen and Gibson 1971, Flavell and Jones 1970), reported values different from Sueoka's for some particular strains, in some cases because of the use of different techniques, in some cases because of the use of strains of questionable provenance (Borden et al. 1973 b). But all workers, regardless of the techniques used, found large strain differences in the DNA composition.

The variation in the G + C% in *T. pyriformis* strains is over twice that found in the vertebrates. If, to take an utterly simplistic view, the base-ratio variation in a group of organisms were directly proportional to the time since they had a common ancestor, we could use the vertebrates, whose age we know, to calibrate the instrument and measure the age of the *T. pyriformis* complex. The vertebrates with 4% variation have a common ancestor about 500 million years ago; an 8% variation in *Tetrahymena* would signify a common ancestor over 1000 million years ago, over halfway back to the origin of the eukaryotes.

The G + C ratio, however, is a crude measure of genetic relationships, and susceptible to unknown perturbances. One needs a more refined measure of molecular similarities. Such a measure, though still with its complications, is provided by DNA–DNA hybridizations. In this procedure the double-standed DNA is "melted" and then allowed to reanneal with DNA from another source. If a foreign DNA is identical to that used as the reference, cross-annealing should be equivalent to reannealing. As differences accumulate, affinities decline and the fraction of reannealing gradually drops. The interpretation of nucleic hybridization data requires some brief discussion. We need to note, for example, that the conditions of reannealing influence the specificity of the results. Moreover repetitive and unique sequences have different hybridization kinetics, and they may have different evolutionary stabilities.

The main comparative hybridization study of total cellular (largely macronuclear) DNA in *Tetrahymena* is that of Allen and Li (1974). They used *T. thermophila* as the reference species and prepared DNA from the other mating species available at the time (Table 8). They separated the repeated from the unique sequences, and these are separately considered. The unique sequences were hybridized first under relatively non-stringent conditions, at 50°C. Obviously, none of the species is close to *T. thermophila*, but they show a gradation; *T. pigmentosa* is relatively closer than the others, even though it shows only about 30% reannealing. This percentage drops sharply when hybridization is carried out at 65°C, when more complete complementarities are required, and the only approximately identical structures fall apart. All the species studied show only about 5% reannealing at 65°.

Table 8

Percentage of DNA reannealing (relative to the control) of various *Tetrahymena* DNA preparations to DNA of *T. thermophila*

Species	Percent of Control Reannealing		
	Repeated Sequences 50°C	Unique Sequences 50°C	65°C
T. thermophila (1)	100	100	100
T. americanis (2)	23, 23	15, 16	
T. borealis (3)	29	21	
T. hegewischi (5)	24	18	
T. canadensis (7)	25	12	
T. pigmentosa (6)	26, 27	29, 29	4.5, 4.7
T. pigmentosa (8)	26	36	6.1
T. tropicalis (9)	25	17	
T. hyperangularis (10)	28	18	
T. australis (11)	22	10	4.2
T. capricornis (12)	21	9	3.1

Allen and Li (1974).

The repeated sequences tell a somewhat different tale. Hybridizations were carried out only at 50°C, and the results are similar for all combinations; all combinations show about 25% reannealing. The degree of complementarity shown by unique sequences is apparently greater (at 50°C) than that shown by the unique sequences. This unexpected result needs further study. Its explanation may lie in highly conserved repeated sequences used in the organization of the macronuclear chromatin. Such a sequence, $(CCCCAA)_n$, has been reported by Blackburn and Gall (1978), and has been used as a probe of macronuclear organization by Yao and Gall (1979). Regardless of precisely how one interprets the limited data available, they unquestionably show that *T. thermophila* has undergone innumerable nucleotide changes since it shared a common ancestor with the other species examined. The simplest explanation is that the species have coexisted for a very long time.

Studies involving bulk DNA have the advantage of assessing the average changes in many molecules. They have the disadvantage of being possibly perturbed by the behavior of atypical or unimportant fractions of the DNA. For this reason one also wishes to examine the molecular divergence of particular molecules of known function. Two such studies are available, on particular DNA molecules of very different evolutionary stability. The first of these is the ribosomal DNA, which specifies the ultraconservative rRNA molecules (excluding the 5S RNA component). The rRNA cistron in *Tetrahymena* occupies a single chromosomal locus in the micronucleus (Yao and Gall 1976). In development of

the macronucleus, the rDNA is released from the chromosome, transformed into a palindrome, trimmed and patched and amplified to a level a couple of orders of magnitude greater than that of other known macronuclear components.

The rDNA's of several *Tetrahymena* species have been analyzed by Din and Engberg (1979). The technique used in this case was restriction enzyme cleavage, which is capable of yielding molecular maps. When several different endonucleases with different sites of action are employed, a reasonably detailed map can be developed, and the maps for molecules of different origin can be compared. The maps now at hand show numerous differences in the rDNA's of the species compared, and show some possibly significant distinctions among the species. Particularly, *T. pyriformis* (*sensu stricto*) and *T. thermophila* are very different from each other and from the other species examined. The latter, though distinguishable, are more similar. Thus, even these very conservative molecules provide evidence of considerable evolutionary distance between the species of the complex even though we are not able to calibrate those distances very precisely.

A last DNA molecule for which we have some comparative data is notoriously labile. The mitochondrial DNA's of the amicronucleate species have been studied by Goldbach et al. (1977), using DNA-DNA hybridization to test for affinities. The results, summarized in Table 9, include comparisons in all combinations, and not just against a single

Table 9

Comparisons of the mitochondrial DNAs of four amicronucleate species of the *T. pyriformis* complex

Species	Reannealing Percentage at 65°C				Molecular Weight $\times 10^{-6}$
	T. pyriformis	*T. elliotti*	*T. furgasoni*	*T. lwoffi*	
T. pyriformis (*sensu stricto*)	100	4	9	4	28.4
T. elliotti	6	100	7	9	31.0
T. furgasoni	11	8	100	5	25.8
T. lwoffi	4	8	5	100	31.0

Goldbach et al. (1977).

reference strain as in the case of the study of Allen and Li. As might be expected for such labile molecules, the cross-annealing values are all very low, in the range of 5–10%. The residual hybridizations probably reflect the conservatism of a portion of the otherwise labile structures — particularly the portion responsible for the ribosomal apparatus of the mitochondrial synthetic system. The mitochondrial data do not help us to order the *Tetrahymena* species in any phylogenetic

pattern; they do confirm our judgement that all the species studied are very far from a common ancestor.

As we have seen, some nucleic molecules are very stable, while others are relatively labile. The same observation can be made for proteins. An example of a very unstable protein, at least unstable for the attribute examined, is the immobilization antigen. Tetrahymenas are readily immobilized by antisera prepared against them (Margolin et al. 1959), and the sensitive immobilization test has facilitated considerable work on the genetics and regulation of molecules involved (Doerder 1979). Thus far, antisera prepared against one species of the *T. pyriformis* complex have never been found to immobilize strains of another species, even at much higher concentrations (Nanney, unpublished, Grass, unpublished). Moreover, antisera prepared against whole cells usually form no sharp bands in heterologous agar diffusion tests. The antigenic properties of the immobilization antigens, and of most other major antigenic molecules, are too unstable to provide estimates of evolutionary distances within the complex.

Very much the same sense of large but unmeasurable molecular distances comes from the electrophoretic analysis of certain enzymes. The esterase enzymes, like the mitochondrial DNAs, are notoriously variable in most organisms and useful chiefly in discriminating among closely related species. Allen and Weremiuk (1971) carried out a survey of the esterases in a number of species of the *T. pyriformis* complex, attempting to describe not only their electrophoretic properties but their specificities. Starting with *T. thermophila*, they defined three classes of esterases (Table 10) on the basis of their substrates and inhibitors.

Table 10

Classes of esterases demonstrable in *Tetrahymena* species. Enzymes noted in parentheses not observed in all strains examined

Species	Esterase-1	Esterase-2	Esterase-3	Summary
T. thermophila (1)	+	+	+	+++
T. americanis (2)	(+)	+	+	+++
T. borealis (3)	+	−	+	+−+
T. hegewischi (5)	−	−	+	−−+
T. canadensis (7)	+	+	+	+++
T. pigmentosa (6)	−	−	−	−−−
T. pigmentosa (8)	−	−	−	−−−
T. tropicalis (9)	+	+	+	+++
T. hyperangularis (10)	−	−	−	−−−
T. australis (11)	(+)	−	+	+−+
T. capricornis (12)	−	+	+	−++

Allen and Weremiuk (1971).

Esterase-1 is a propionyl esterase, activated by sodium taurocholate and inhibited by eserine sulfate. Esterase-2 is a butyryl esterase activated by p-chloromercuribenzoate and insensitive to eserine. Finally esterase-3 acts on both α-naphthyl propionate and butyrate; it is activated by both sodium taurocholate and p-chloromercuribenzoate and is insensitive to eserine. These specifications seemed suitable for identifying the arrays of isozymes in the various species.

However, the esterases differ not only in their electrophoretic mobilities but also in their other enzymatic characteristics. Three species manifested isozymes of all three classes, but in the other species one or more of the classes is missing entirely. *T. pigmentosa* and *T. hyperangularis* have no enzymes that can be unequivocally associated with esterases-1, -2, or -3. These observations do not prove that homologous proteins are missing from the other species, or even that the homologous proteins are no longer esterases. They do demonstrate that some of the functional characteristics of these enzymes are labile within the complex, despite the superficial physiological and nutritional similarities of the organisms.

Simply to show that the variability of the esterases is no isolated example, consider for a moment the results of a recently published (Nanney et al. 1981) starch gel electrophoretic analysis of the NADP-isocitrate dehydrogenase enzymes. *T. pigmentosa* (Table 11) manifests three isozymes — a proximal, a middle and a distal band. Other species show one, two, or three bands, and their positions within the general region in which they appear are variable. We can arbitrarily classify a species as to the isozymes present (or absent) within each region of the gel, and as to their relative mobility (fast or slow). Although this procedure provides "characters" that can be used in sorting the species, we cannot be confident of the genetic relationships of the molecules being compared. This enzyme may be somewhat more evolutionarily constrained than are the esterases, but not much more. The enzyme's lability is too great for it to be used as a reliable measure of evolutionary distance in species so distantly removed from a common ancestor. This same conclusion is probably appropriate for isozymic studies on several other enzymes (Borden et al. 1977); in many cases one simply cannot compare the electrophoretic mobility of enzymes for two species, because the enzymes are too different to be visualized under the same circumstances on a gel. Such data may be helpful for taxonomic purposes, in the identification of a species; they are probably useless in defining phylogenetic relationships.

Primarily because the form of *Tetrahymena* is so perfectly preserved in all the species of the complex, one might suppose that structural proteins would be more conservative in this group than are many of the

Table 11

Relative electrophoretic mobilities of isocitrate dehydrogenase (IDH) isozymes in *Tetrahymena* species. Symbols in parentheses indicate weak and irregularly observed bands. F refers to fast migrating bands, S to slowly migrating bands

Species	Proximal Band		Middle Band		Distal Band		Summary
	5–10	11–17	45–55	56–68	99–110	111–123	
Amicronucleate species							
T. pyriformis (A)				+		+	OFF
T. elliotti (B)			+		+		OSS
T. furgasoni (C)			+				OSO
T. lwoffi (E)			(+)				OSO
Micronucleate species							
T. thermophila (1)			+		+		OSS
T. americanis (2)	+				+		SOS
T. borealis (3)	+				+		SOS
T. hegewischi (5)		+		(+)	+		FFS
T. canadensis (7)	(+)			(+)		+	SFF
T. pigmentosa (6)	+			(+)		+	SFF
T. pigmentosa (8)		+		+		+	FFF
T. tropicalis (9)	(+)			(+)	+		SFS
T. hyperangularis (10)	(+)				+		SOS
T. australis (11)	(+)				+		SOS
T. capricornis (12)						+	OOF
T. sonneborni (13)		+	+		+		FSS
T. nipissingi (14)		+		+	+		FFS

Nanney et al. (1981).

catalytic proteins. Indeed some structural proteins are very conservative, at least in some respects. Vaudaux et al. (1977) extracted the major cortical proteins of a number of species of *Tetrahymena* and separated them according to molecular weight (Table 12). All the species, including two of the *T. patula* complex, had a protein of high molecular weight of about 250 000 daltons. All species also had one or two smaller proteins, falling into a limited number of molecular weight classes. The patterns yield to no simple evolutionary scheme, primarily because of the similar patterns expressed within and outside the species complex. Obviously the system of proteins is highly constrained with respect to molecular weight classes, but the architectural rationale or the evolutionary basis of the constraint is obscure. Species with the same patterns are not necessarily more closely related.

Another study of structural proteins also gives evidence of some unexpected molecular conservatism in the face of large scale variability. Seyfert and Willis (1981) extracted the proteins of the cilia of five species of the *T. pyriformis* complex and studied their molecular weights by SDS-polyacrylamide gel electrophoresis. Over 30 polypeptides

Table 12

Comparison of major structural proteins of the *Tetrahymena* cortex

Species	Molecular Weight × 10^{-3}						Pattern Class
	122	140	145	155	174	250	
T. pyriformis complex							
Amicronucleate species							
T. pyriformis (A)	–	+	–	+	–	+	I
T. elliotti (B)	–	–	+	–	–	+	IV
T. furgasoni (C)	+	–	–	–	+	+	II
Micronucleate species							
T. thermophila (1)	+	–	+	–	–	+	V
T. americanis (2)	–	–	–	+	–	+	III
T. borealis (3)	+	–	–	–	+	+	II
T. hegewischi (5)	–	–	–	+	–	+	III
T. canadensis (7)	+	–	–	–	+	+	II
T. pigmentosa (6)	–	–	–	+	–	+	III
T. pigmentosa (8)	+	–	–	–	+	+	II
T. tropicalis (9)	+	–	–	–	+	+	II
T. hyperangularis (10)	–	–	–	+	–	+	III
T. australis (11)	–	+	–	+	–	+	I
T. capricornis (12)	–	–	–	+	–	+	III
T. patula complex							
T. patula (L-FF)	–	–	–	+	–	+	III
T. vorax (Tur)	–	+	–	+	–	+	I
(V_2S)	–	–	+	–	–	+	IV

Vaudaux et al. (1977).

were examined and only two had identical molecular weights across the few species examined. In comparison of any two species the percentage of bands with identical molecular weights was less than 65%. The authors suggest that one of the constant proteins is probably tubulin, but they point out that similarity of molecular weight by no means implies identity of primary structures in this or any other molecule. In an interesting cognate study these authors prepared antisera against ciliary preparations, and were unable to obtain interspecific cross reactions in agar diffusion systems.

I will mention only one additional class of structural proteins, one which should under any view be especially conserved. The ribosomal proteins, like the ribosomal RNA's, trace their ancestry back to the progenote, and they help conserve the rigid grammatical rules of information transfer. Recently Cuny et al. (1979) carried out a two-dimensional electrophoretic comparison of two *Tetrahymena* species, *T. pyriformis* and *T. thermophila*, and detected many differences in their ribosomal proteins (Table 13). Only nineteen of the 34 or 35 basic proteins of the large subunit were indistinguishable. Only 7 of the 9 or 11 acidic

Table 13

Two-dimensional electrophoretic comparison of ribosomal proteins of two *Tetrahymena* species

Species	Basic Proteins		Acidic Proteins
	Small Subunit	Large Subunit	
T. pyriformis	35	40	11
T. thermophila	34	38	9
Comigrating	19	21	7

Cuny et al. (1979).

proteins were alike. At least 34 of the ribosomal proteins in the two species were different in either charge or molecular weights. Differences in sequence would undoubtedly be even more widespread.

(5) The Combination of Phenetic Similarity and Genetic Diversity in the *T. pyriformis* Complex Provides a Powerful Tool in Comparative Biology

The simplest explanation for the observations summarized here is that the *T. pyriformis* complex is very ancient; not only was the ancestral ciliate derived very early from the eukaryotic root, but different species of the *T. pyriformis* complex diverged from each other genetically at some very ancient but still imperfectly measured time. The phenetic constancy of the complex suggests that certain of its features have been stringently selected so as to maintain them unchanged, or within narrow limits, since the remote time of their common origin. The "organismic design" of the *Tetrahymena's* shares some evolutionary similarities to other complex organic designs, as in the ribosome, the cilium and the nucleosome, that were brought to a kind of perfection long ago and maintained thereafter through stabilizing selection. An interesting feature in all these cases is the suggestion that perfect molecular conservation is not required for the preservation of the essential features of complex organic constructs. Many kinds of molecular changes are consistent with the perfectly adequate functioning of complex organelles.

An opportunity for comparative biology lies in the array of multiple evolutionary replicates of a common design. We are permitted to ask which features of a design are truly essential and which are merely circumstantial. Who would have thought that the unusual palindromic structure of *Tetrahymena's* ribosomal DNA would be preserved, even though the nucleotide sequences and the lengths of the cistrons vary

considerably among the species? Who would guess in advance that the atypical linearity of the mitochondrial DNA would be found in all the *Tetrahymena* species, even when their sequences are scrambled beyond recognition? Why is the molecular weight of tubulin critical to the structure of the cilium? How can one account for the invariance of the molecular weight of the large cortical protein? What is so special about the karyotype of *Tetrahymena*, that its five pairs are preserved even when the base ratios of DNA shift by 8–10%? What is so significant about the size of *T. pyriformis*? Why can't tetrahymenas grow as large as paramecia? Why does the contractile vacuole pore open asymmetrically on the side of the cell? on the right side? at 25% of the distance around the cell? How does one explain the constancy of nutritional requirements in all the species?

Our claim is, in essence, that *Tetrahymena* is a kind of living fossil, an organism frozen in its organismic characteristics for a long, long time. Organisms fixed in their attributes have a peculiar fascination, and the longer they have persisted in their invariant adaptations the more fascinating and useful they become. The opossum, *Didelphus*, seems organismically unchanged for 75 million years, the coelocanth, *Latimeria*, for 100 million, the horseshoe crab *Limulus* is 200 million years old. Conceivably, *Tetrahymena* is 5 or even 10 times as old as that. And we have available not one surviving species, but many, natural replicates of evolutionary experiments in stabilizing selection.

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Symposium C

Membrane Fusions in Protozoa. A Survey Lecture with Special Emphasis on Exocytosis in Ciliates

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General Remarks

As in metazoan cells, membrane fusion events are involved in a variety of processes also in protist cells (see Fig. 1, see also Allen 1978 a). Among them one can list the discharge (exocytosis) or uptake (endocytosis) of molecules or bulk materials (pino- and phagocytosis), the insertion and recycling of membrane components, fusion and fission processes between certain intracellular compartments (e.g., lysosomes and phagosomes etc.), certain osmoregulatory functions and the fusion of cells (mating reactions). Some protozoa have at their disposition a highly efficient and sophisticated "machinery" for membrane fusion-fission processes. Such structures may be permanently "installed" either at operationally favourable and, eventually in high redundancy, at precisely predictable sites. This is in contrast to most (not all) metazoan cells and should facilitate correlated structural and functional analyses. This is also the main reason why ciliated protozoa, notably *Tetrahymena* and *Paramecium*, became important model systems for the study of membrane fusion events, particularly for the study of exocytosis; another reason is the unique occurrence of exocytotic mutations in paramecia.

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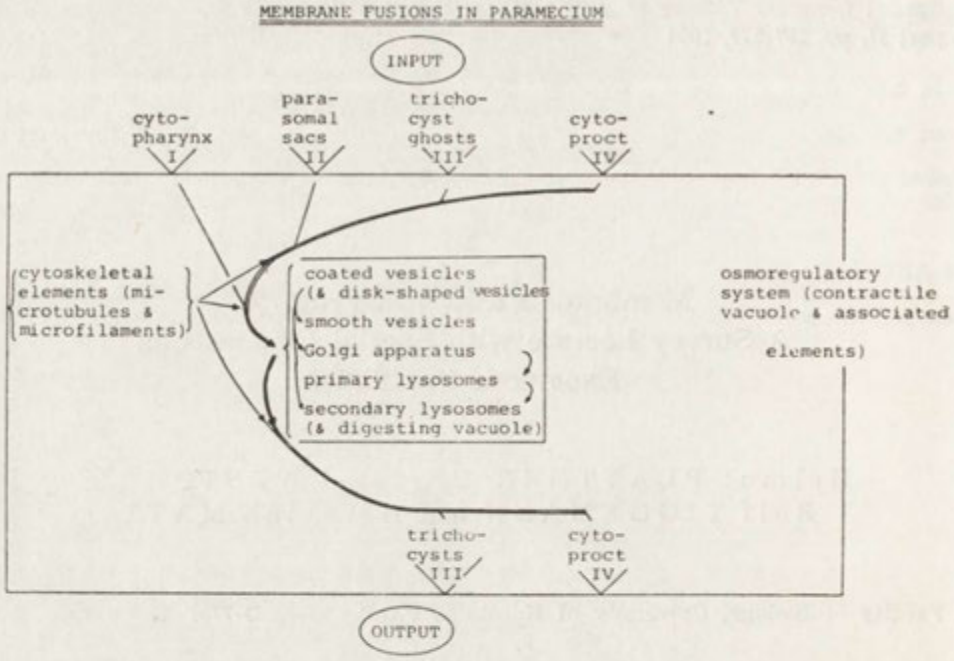

Fig. 1. Survey of membrane fusions in a ciliated protist (*Paramecium*). There are many sites of in- and output of materials which all require membrane fusions and frequently the interaction with cytoskeletal elements. Some of the sites serving material in- and output are involved in membrane recycling. (The frame in the middle gives only a rough outline of intracellular membrane interactions; for more details see R. D. Allen, these proceedings (Part I)). Osmoregulatory systems appear to be quite independent, with regular membrane fusions at their outlet on the cell membrane

Of course, one has to analyze in due time to what extent a certain model allows for conclusions of more general validity (see below).

Exocytotic System in Ciliates

It was noted independently by different authors using the freeze-fracture technique that potential exocytotic fusion sites in *Tetrahymena* (Satir et al. 1972, 1973, Wunderlich and Speth 1972) and *Paramecium* (Janisch 1972, Bachmann et al. 1972) display highly ordered arrays of membrane-integrated particles (MIP). *Tetrahymena* contains a $\sim$ 50 nm large "rosette" of $\sim$ 10 MIP within the cell membrane precisely at the sites where mucocysts are discharged. In *Paramecium* the emphasis was first on the occurrence of a $\sim$ 300 nm large double "ring" of MIP which surrounds the potential fusion site, but "rosettes" are also present right in the center of each "ring" (Plattner et al. 1973, Beisson et al. 1976).

It is difficult to derive a time sequence or — even more — any possible functional implications of static snap shots one gets from membranes by cryofixation and freeze-fracturing. This holds even more if any such attempt is done after previous chemical pretreatments (aldehyde fixation, antifreeze impregnation). In retrospect it appears, as summarized by Plattner (1981), that much of the controversies (see below) between different groups, working with protozoa, and also other groups, working with metazoan systems, came from such preparative difficulties.

Both *Tetrahymena* and *Paramecium* display "rosettes" already in the resting stage, i.e., without triggering. After exocytosis triggering by a Ca^{2+}-ionophore, "rosettes" are no longer present, whereas the "rings" persist and collapse (Plattner 1974). This indicates that only "rosette" but not "ring" structures are directly involved in exocytosis performance; the "rings" merely delineate the potential fusion zones without participating in the fusion process. Even stronger evidence for this comes from freeze-fracture work with mutant strains of *Paramecium tetraurelia* (Beisson et al. 1976). A brief survey is given in Figs. 2 and 3. Some mutations make no (*tl*) or grossly defective (*ftA, tam* 38) trichocysts which cannot be attached to the cell membrane. Other mutations (*nd*) allow the attachment but not the subsequent exocytotic discharge. Beisson et al. (1976) established that the discharge capacity is coupled with the presence of "rosettes". This has later been extended to further mutations (Beisson et al. 1980, Lefort-Tran et al. 1981).

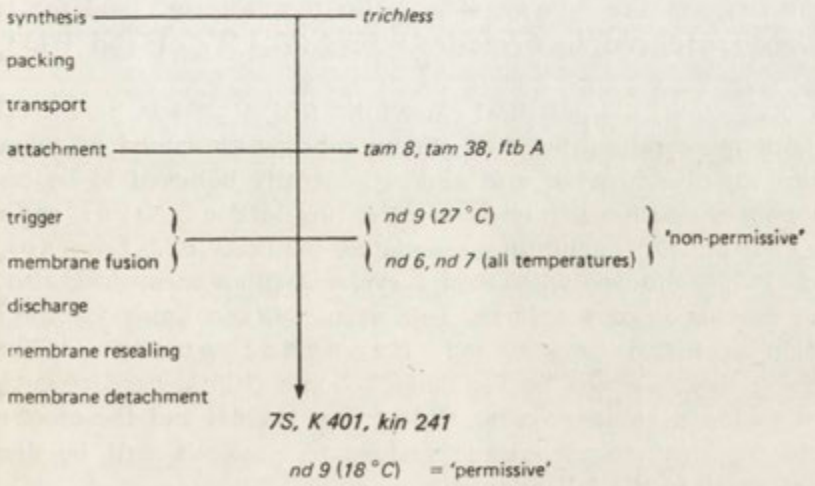

Fig. 2. Sequence of events leading to exocytotic membrane fusion (left) and genetic disturbances at different levels in various mutant strains of *Paramecium tetraurelia*. *7S* is the wild type strain (*K401, kin 241* are non-exocytotic mutations); for *nd 9* see text. From Plattner et al. (1980)

Some time ago we have documented the occurrence of some ill defined, electron dense "connecting material" between trichocyst and cell membrane (Plattner et al. 1975, 1977). This material has now been further characterized by group- and charge-specific electron stains in conjunction with enzymatic extraction experiments (Westphal and Plattner 1981 a, b; see Fig 4); it represents protein(s) with a surplus of negative charges. Similar analyses with *nd* 9 cells grown under permissive or non-permissive conditions, revealed that the simultaneous presence (or absence) of "connecting material" (and of "rosettes"; see above) is correlated with the (un-) capability for exocytosis performance (Beisson et al. 1980, Matt et al. 1980, Plattner et al. 1980). We have chosen the term "connecting material" because of our ignorance on its precise nature and function (for possible speculations see below) and to indicate that it connects physically the trichocyst and cell membrane (selectively in those strains which are capable of membrane fusion: Plattner et al. 1980); this holds for a variety of exocytosis trigger procedures (Matt et al. 1980). Furthermore, microinjection studies using exocytotically normal and defective *Paramecium tetraurelia* strains as donors and/or receptors gave independent support for the functional importance of a kind of "connecting material" (Beisson et al. 1980).

Table 1 summarizes some of the features relevant for exocytotic membrane fusion in *Paramecium tetraurelia*.

Not only the "connecting material" between trichocyst and cell membrane is sensitive selectively to proteolytic enzymes (Westphal and Plattner, 1981 a, b; Fig. 4) but also the "rosette" (and the "ring") MIP when analyzed by freeze-fracturing (Vilmart and Plattner 1983).

In conclusion, the potential exocytotic fusion sites in paramecia contain both membrane-integrated and membrane-associated proteins. This is quite opposite to what was almost generally believed to be essential for membrane fusion in metazoan cells (for MIP c.f. Orci and Perrelet 1978; for membrane associated material c.f. Lawson and Raff 1979). Studies with model systems (liposomes) had also come to the conclusion of a primary role of membrane lipids for the determination of fusion capacity (c.f. Papahadjopoulos 1978). How can these discrepancies be reconciled? Some points were recently discussed within a broader frame (Plattner 1981) and the most crucial aspects pertinent to membrane fusions in protozoa will be discussed in more detail in the following.

What might be the functional role of this assembly of membrane-integrated and membrane-attached proteins for membrane fusion? With certain expectations in mind we localized a Ca^{2+}-stimulated ATPase

Table 1

Characterization of exocytotic membrane fusion in *Paramecium tetraurelia* strains*

Strain	Trichocyst attachment to cell membrane	Exocytosis capacity	Freeze-fracture		Ultrathin sections			Biochemistry (isol. pellicles) $V_{max}(Ca^{2+}$-ATPase)	Matrix stretching in vitro	Ciliary reversal upon triggering
			"rosettes"	"rings"	"connecting material"	Ca^{2+} ATPase	p-NO_2-phenylphosphatase			
7 *S*, K401 *nd* 9 (18°C)	+	+	+	+	+	+	+	higher	+	+
nd 6, *nd* 7 *nd* 9 (28°C)	+	–	–	+	–	–	–	lower	+	+
tam 38, *tl* (*trichless*)	–	–	–	+**	(–)	–	–	lower	–	+

* Data pooled from Beisson et al. (1976, 1980), Bilinski et al. (1981), Lefort-Tran et al. (1980), Plattner et al. (1973, 1975, 1977, 1980), Matt et al. (1980), Westphal and Plattner (in press) and Beisson et al. (unpublished results).

** In form of "parentheses".

activity (Fig. 5) at the preformed exocytotic fusion sites of paramecia (Plattner et al. 1978). This activity was absent from exocytotically inactive mutations devoid of "rosettes" and simultaneously of "connecting material" (Plattner et al. 1980); see Table 1. Some differences were also found biochemically, when pellicles were isolated from exocytosis-capable and -incapable strains and assayed by spectral photometric tests for their Ca^{2+}-ATPase activity (Bilinski et al. 1981 b). All this opens up several possibilities: The ATPase could represent a Ca^{2+}-pump (keeping the local Ca^{2+} concentration low and thus avoiding membrane fusion in the untriggered state; see also Beisson et al. 1980), a site of protein phosphorylation (as in other exocytotic systems, like synaptosomes), a Ca^{2+}-channel (if combined with a pump, as in other systems, like sarcoplasmic reticulum) or it could indicate the presence of contractile elements of the actomyosin type. Since exocytosis occurs in response to ATPase inhibitors (Matt et al. 1980), the pump function appears possible, although we have no definite proof for this assumption. Protein phosphorylation would also be compatible with our cytochemical findings (Plattner et al. 1977, 1980) but this interpretation would still require biochemical confirmation. A Ca^{2+} channel function was inferred by Satir and Oberg (1978) but it was shown later, that an alternative interpretation of their results would be possible (Matt et al. 1980), although their concept could be correct. As to contractile elements, it was shown by immunofluorescence (rabbit-anti (*Paramecium*) actin IgG) and affinity fluorescence (DNase I, heavy meromyosin) labeling (Tiggemann and Plattner 1981) as well as by immuno-electron microscopic methods (Tiggemann et al. 1981) that the potential fusion sites proper, i.e., the "connecting material" regions, are devoid of actin (Plattner et al. 1982; see Fig. 6). This questions any direct interference of actin (or microfilaments, respectively) in the membrane fusion process — in opposition to earlier assumptions along these lines (Poste and Allisson 1973).

In conclusion we assume that our cytochemical findings could indicate most likely the local presence of a Ca^{2+}-dependent ATP-splitting activity, possibly combined with a Ca^{2+}-transport function (pump and channel?). "Rosettes" and "connecting material" occur always together and only in exocytosis-capable *Paramecium* strains. This could be seen as being relevant for the structural assembly of the "rosette" MIP (which would be held together by the apposed "connecting material") and probably as being also functionally relevant, e.g., as a combined enzyme-activator type system (see discussion in Plattner et al. 1980). As to the latter possibility it would be rewarding to test for the presence of calmodulin or related proteins within the structural component with

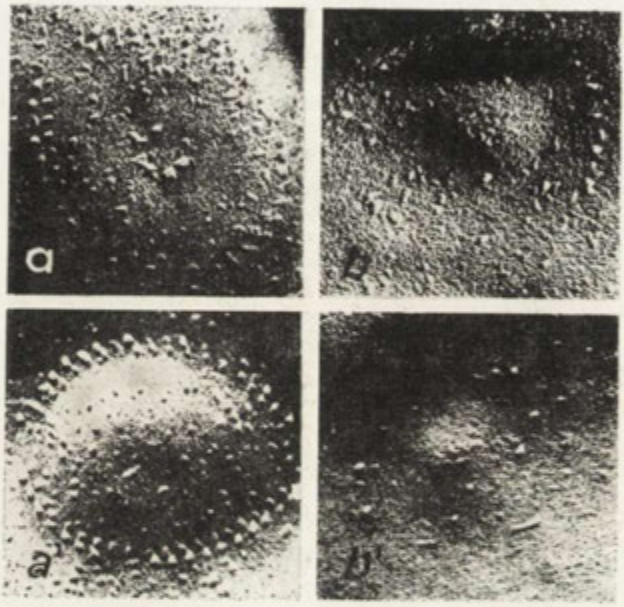

Fig. 3. Freeze fractured trichocyst attachment sites (cell membrane) of *Paramecium tetraurelia* cells, strain *nd 9*. (a, b): *nd 9* grown at the permissive temperature of 18°C, a — representing the plasmatic fracture face (P-face), b — showing the exoplasmic fracture face (E-face). Note the occurrence of a "ring" and of a "rosette" of MIPs in one of the fracture faces (P-face). (a', b'): *nd 9* cells grown at the non-permissive temperature of 27°C are devoid of a "rosette" on both fracture faces (a' — P-face, b' — E-face). 100 000×. From Beisson et al. (1976) with kind permission of the author

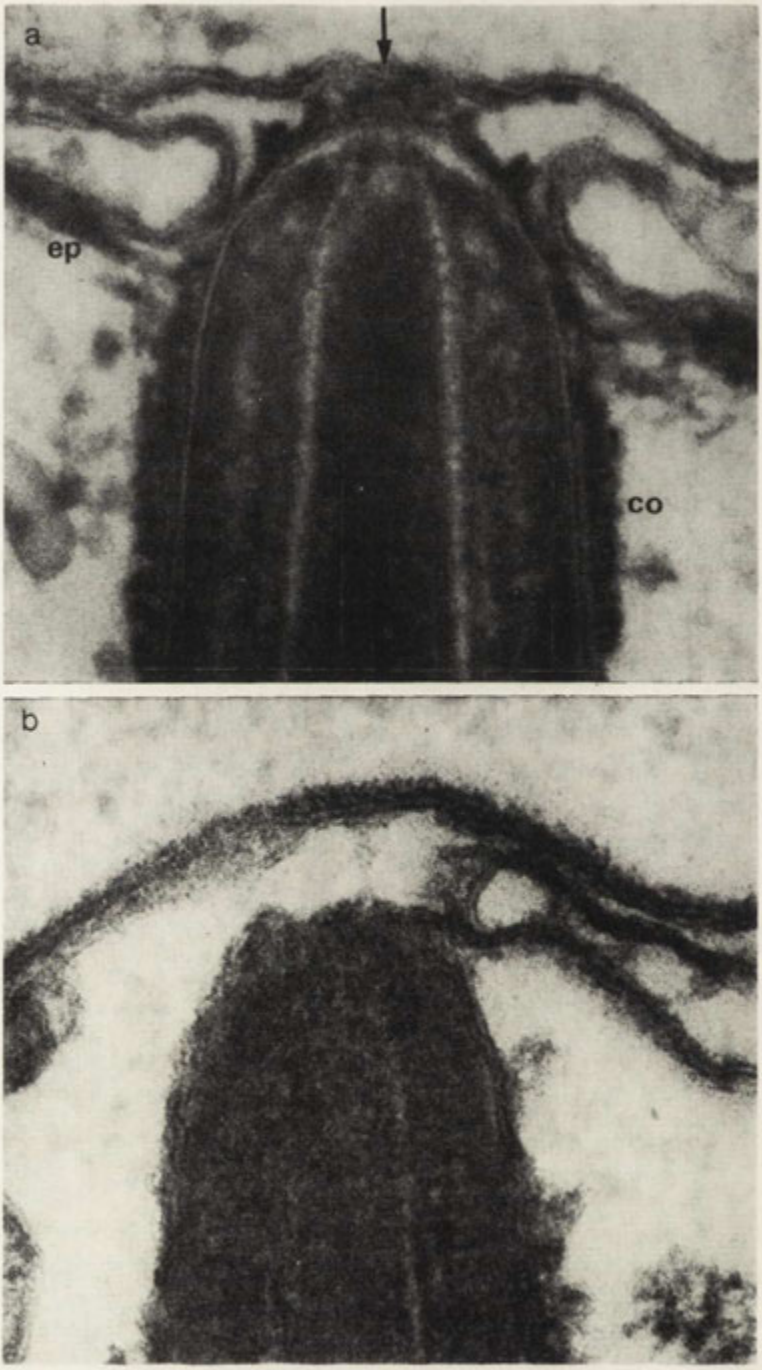

Fig. 4. Trichocyst attachment site of an exocytotically normal *Paramecium tetraurelia* cell (strain *K401*), as seen on an ultrathin section after tannic acid staining. a — Normal situation showing the presence of "connecting material" (arrow) between trichocyst and cell membrane. "co" designates the collar, "ep" the epiplasm, b — In a pronase treated aliquot all these structures are digested. 220 000 ×

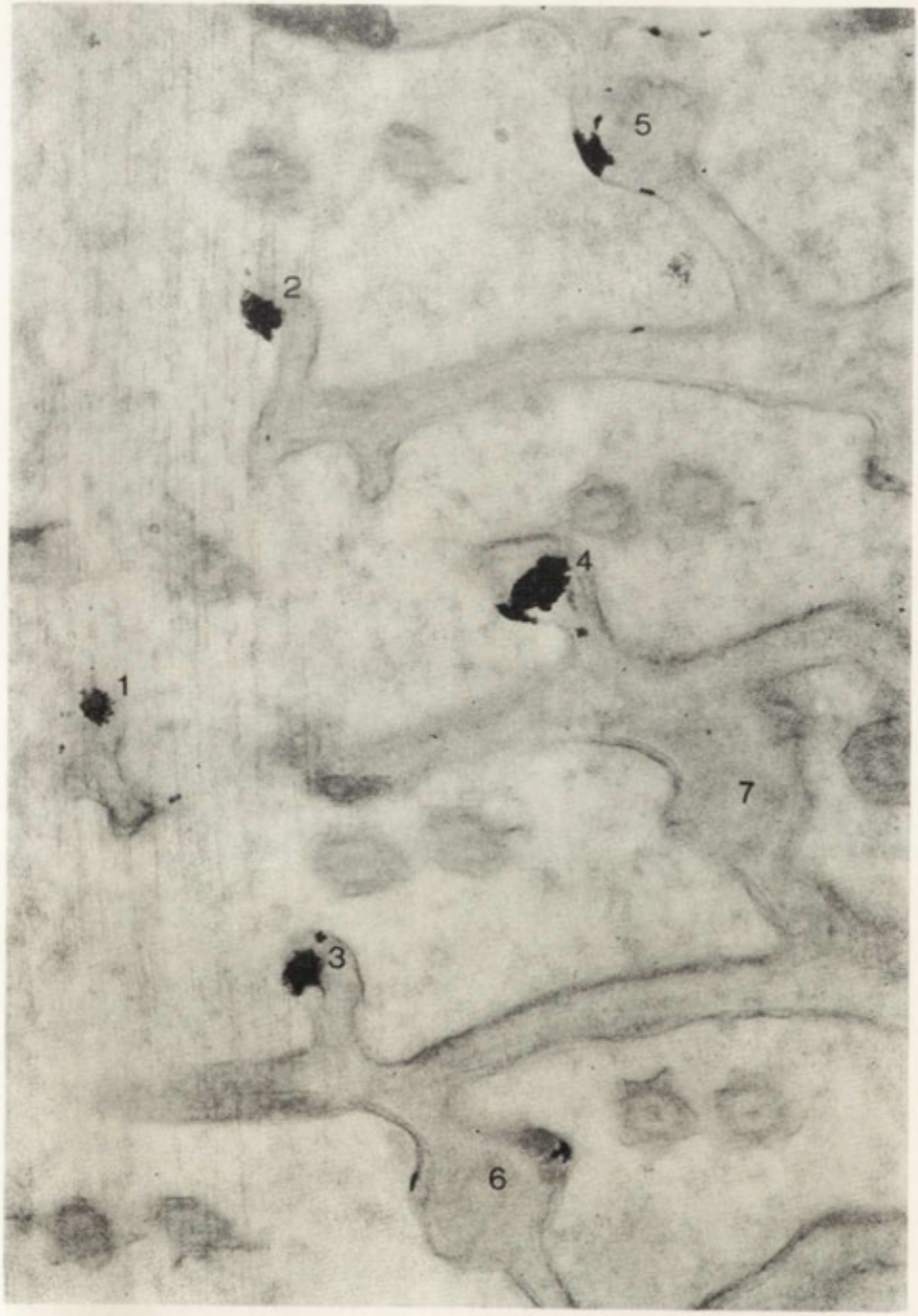

Fig. 5. Tangentional section through the surface of an exocytotically normal *Paramecium tetraurelia* cell (strain 7*S*) after performance of a Ca^{2+}-ATPase reaction. Only the trichocyst attachment sites are positive; numbers 1–7 indicate different sectioning levels (6 and 7 being negative because of being cut at too low a level). 39 000×. From Plattner et al. (1980)

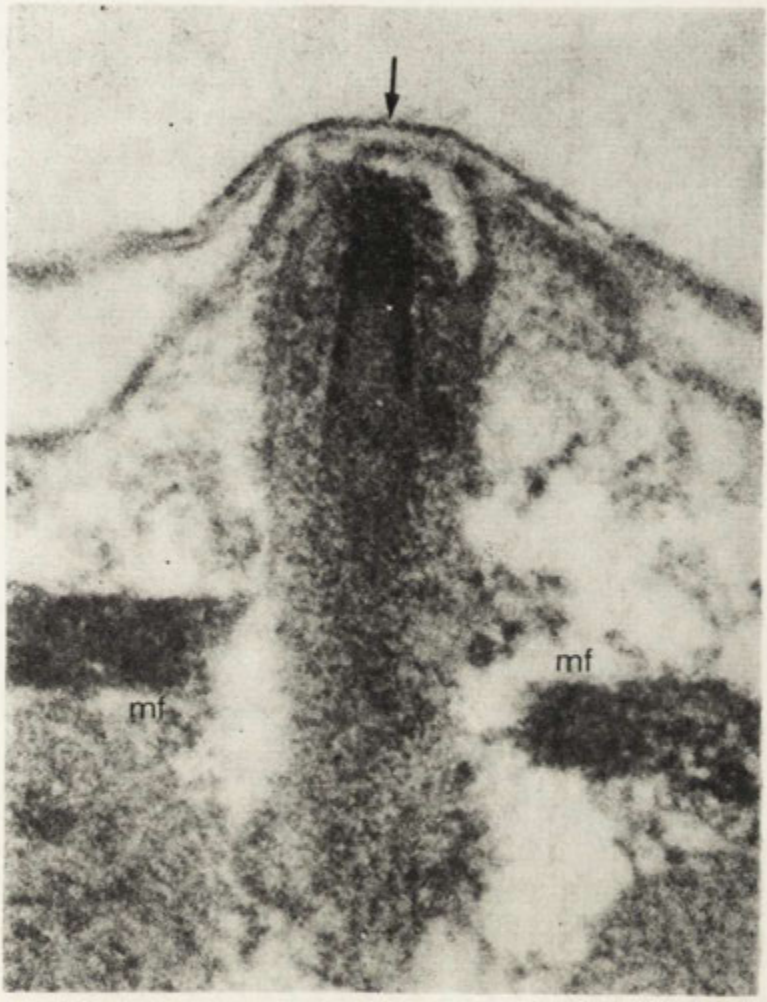

Fig. 6. Ultrathin section through a trichocyst attachment site of a *Paramecium tetraurelia* (*K 401*) cell after immunocytochemical staining for actin, using rabbit anti-(*Paramecium*) actin IgG coupled to horseradish peroxidase (type VII) for visualization by the diaminobenzidine technique. Only the microfilaments (mf) which surround the trichocyst tip are reactive; the trichocyst attachment site on the cell membrane (arrow) is negative. (The "inner lamellar sheath" within the trichocyst tip displays some endogenous electron density). 100 000 ×. From Plattner et al. (1982)

the preliminary designation "connecting material". Such attempts have been started just recently.

It deserves to be mentioned that the uppermost trichocyst tip (adjacent to the cell membrane) is always devoid of MIP; the "annulus" MIPs surround the fusion site proper somewhat below, so that this structure (like the "rings" within the cell membrane) seems to be situated outside the fusion zone proper.

Exocytotic Systems in Other Protists and in Metazoa

"Rosette" type MIP clusters (without a "ring") have been observed also over extrusomes of heliozoa (Davidson 1976) and sporozoa (*Eimeria*: Dubremetz and Torpier 1978, *Toxoplasma*: Porchet and Torpier 1977: *Plasmodium*: Dubremetz et al. 1979, Bannister, personal communication). In the case of sporozoa exocytotic events are believed to participate in the host cell penetration (*Eimeria*: Jensen and Edgar 1976; *Toxoplasma*: Nichols and O'Connor 1981). During conjugation of the ciliate *Euplotes* the contents of ampullae are released by exocytosis and again "rosettes" were found at these sites (Luporini, personal communication). Nothing alike was reported for amoebae (for *Entamoeba* see Henley et al. 1976). There are so far no data available on the occurrence of "connecting material" in these cases.

Among metazoa, exocytotic sites are characterized by conspicuous MIP aggregates only in motor endplates (c.f. Meldolesi et al. 1978). For many other systems it was claimed that MIPs would be removed from the fusion sites on a large scale before fusion (Orci and Perrelet 1978). This view has recently been challenged for methodical reasons (Plattner 1981). Careful freeze-fracture experiments with various gland cells also showed that exocytotic fusion events in several mammalian gland cells might occur without any or with only a small MIP shift (Tanaka et al. 1980), so that MIPs might here also be located quite closely to the fusion zone. We arrive to the following conclusion. If some membrane (integrated and/or associated) proteins would stay close to the fusion site, they could exert a positive modulatory effect, e.g., by exerting a Ca^{2+}-channel function as mentioned above or by changing the fusogenic properties of the tightly apposed membranes (to mention just two possibilities). Supporting evidence along these lines came recently from work with liposomes which fuse in the presence of physiological Ca^{2+} concentrations only when supplemented with certain proteins (Zimmerberg et al. 1980), whereas previous work on lipo-

some fusion concentrated entirely on the physical effects on lipids (c.f. Papahadjopoulos 1978). It deserves also considerable interest now that membrane associated proteins were found more and more also on the contact sites of secretory organelles and plasmalemma in metazoan systems (for review see Plattner 1981).

All this would imply that proto- and metazoan systems would not be principally different with regard to membrane fusion processes, but that protozoa would contain the same elements in an amplified form. One could further speculate, that regular MIP arrays and apposed "connecting material" would have to be present, wherever a membrane fusion event would have to take place precisely at a certain site. This assumption would hold not only for the above mentioned cases in protozoa but again also for some metazoan systems (e.g., neuromuscular junctions, where transmitter molecules are released rapidly and precisely over underlying junctional folds; c.f. Meldolesi et al. 1978). Further examples and arguments along these lines were presented elsewhere (Plattner 1981). Another example for the simultaneous occurrence of MIP arrays and membrane-attached materials in fusogenic zones of protist cells would be the mating structures of *Chlamydomonas* (see below).

Other Fusion Sites in Protists

The theory proposed above could also account for the occurrence of MIP aggregates in mating structures (*Chlamydomonas*: Weiss et al. 1977 a; *Euplotes*: Luporini, personal communication). Regular MIP arrays were reported also from the *Paramecium* cytoproct and contractile vacuole outlet (Allen 1978 b), where they were thought, however, to reflect the pattern of underlying microtubules rather than being involved in membrane fusion events. MIP aggregates are also found around the contractile vacuole outlet of *Chlamydomonas* (Weiss et. al. 1977 b). For other membrane fusion events in protists see also the review articles by R. D. Allen (1978 a and these proceedings, Part I).

Possible Changes During Fusion

The actual fusion process is now assumed to be a rather focal event (Plattner 1981, see Fig. 7); quite a similar fusion model was recently put forward also for artificial lipid membranes (Hui et al. 1981). Also for this reason large intramembraneous MIP shifts before fusion would not be required, although it appears likely that the actual fusion site would be merely lipidic. It could be represented by a small focal pertur-

bation of lipids which then would be reorientated to make adjacent membranes continuous. MIPs could then secondarily spread in lateral direction around the expanding exocytotic canal. Data from Haus-

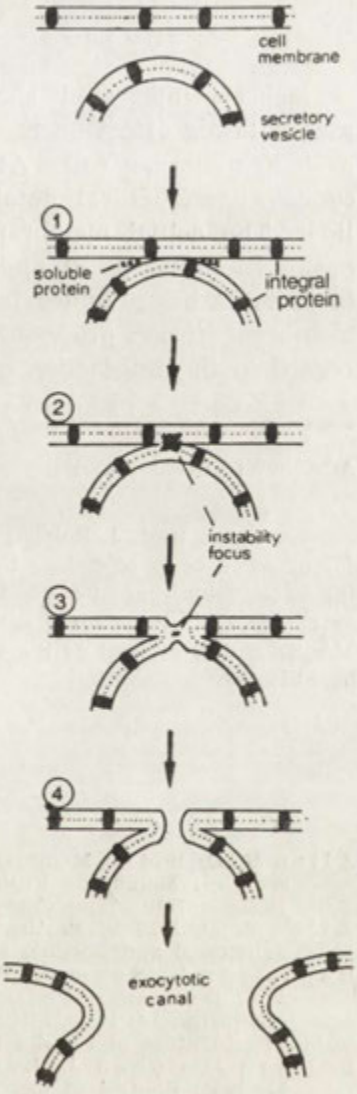

Fig. 7. "Focal membrane fusion model" according to Plattner (1981). It indicates that membrane-integrated and membrane-associated proteins remain closely associated with the fusion site (so that they can exert a modulating effect). This model also assumes that lipids fuse by rearrangement within a very small focus and that the exocytotic canal expands later on. From Plattner (1981)

mann and Allen (1976) indicate that this would involve only little — if any — intermixing of components from both membranes involved. It also appears relevant in this context that the membrane fusion events involved in the discharge of trichocysts appear quite independent from the events in the matrix space; the trichocyst matrix is decondensed

(stretched and shot out) by exogenous Ca^{2+}, once Ca^{2+} can come in via the already formed exocytotic canal (Bilinski et al. 1981 a).

Conclusions

Mebrane-integrated and membrane-associated proteins may exert a modulating effect on membrane fusion in ciliates, other protozoa and even in metazoan cells. At least some of these systems display conspicuous ultrastructural details which could be interpreted along these lines. The actual membrane fusion appears to be a focally restricted event. In retrospect it appears now that research on membrane fusion in protozoa has provided important clues for a general understanding of membrane fusion processes. Such research appears also important with regard to the interaction of protozoan parasites and host cells.

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Zimmerberg J., Cohen F. S. and Finkelstein A. 1980: Micromolar Ca^{2+} stimulates fusion of lipid vesicles with planar bilayers containing a calcium-binding protein. Science, 210, 906–908.

LIST OF SCIENTIFIC REPORTS
presented at Symposia, Contributed Paper and Poster Sessions

The aim of the editors of Post-Congress Volume of "Proceedings of VI International Congress of Protozoology" was to prepare extensive summaries of all Symposia and Contributed Paper Sessions, which were organized during VI International Congress of Protozoology in Warsaw (July 5–11, 1981). Unfortunately, only few summaries were submitted by contributors and under these conditions we would publish only the list of Scientific Reports presented at Symposia Contributed Paper and Poster Sessions with information concerning designation of session, the number of abstract etc. as indicated in Pre-Congress Volume (Progress in Protozoology — Abstracts of papers submitted to VI International Congress of Protozoology) and Post-Congress Volumes (Progress in Protozoology, Proceedings of VI International Congress of Protozoology, Special Congress Volume of ACTA PROTOZOOLOGICA, Part I and Part II).

Symposium A and Contributed Paper Session, Section I: Variation, Life Cycles, Systematics and Phylogeny of *Protozoa*

Chairman: Jean Génermont, Université Paris-Sud, Paris, France
Co-Chairmen: Emil Vivier, Université de Lille, Lille, France
Kristian Bardele, University of Tübingen, Tübingen, FRG
Convener: Anna Czapik, Jagiellonian University, Kraków, Poland

D. L. Nanney (USA), Intraspecific and interspecific variation within the *Tetrahymena pyriformis* complex
abstr. 266 and proc. II 243–266

Contributed papers

D. K. Chatterjee (India), Life-cycle of *Trypanosoma avium bakeri* and its host-specificity abstr. 54

V. Golemansky (Bulgarie), Origine, relations philogenetiques et évolution des Thécamoebiens interstitiels du supralittoral marin abstr. 117

E. Vivier (France), Examen des critéres taxonomiques utilisables chez les *Sporozoa-Apicomplexa* et propositions systématiques abstr. 382

Z. Černa (Czechoslovakia), Further studies on the life cycle of sarcosporidians in the intermediate host abstr. 49

P. J. O'Donoghue, E. Goebel (FRG), The schizogenous proliferation and tissue cyst development in sheep of *Sarcocystis* sp. from dogs; as revealed by light and electron microscopy abstr. 276

J. Lom, I. Dyková (Czechoslovakia), Biological and morphological features of two myxozoans of the genera *Sphaerospora* and *Mitraspora* infecting carps abstr. 222

H. K. Hinaidy (Austria), Taxonomie und Morphologie von *Babesia divergens* abstr. 138

U. G. Moltmann, H. Mehlhorn, E. Schein, W. P. Voigt (FRG), Fine structure of supposed sexual stages of two *Babesia* species (*Piroplasmia*) after *in vitro* development abstr. 256

J. P. Mignot (France), Evolution des systèmes microtubulaires au cours du cycle de reproduction chez l'Heliozoaire *Actinophrys sol* abstr. 251

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D. H. Lynn, B. R. Oakley (USA), *Mesodinium rubrum*: ultrastructure and systematic position abstr. 226

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C. Demar (France), Intrageneric acid-phosphatases variability in *Euplotes* (*Ciliata, Hypotrichida*) abstr. 73

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D. Mandal, A. Choudhury (India), Studies on the coccidian parasites of wild gaur *Bos gaurus* (Indian "bison") of Betla Forest, Palamuo Tiger Reserve, India abstr. 229

L. H. Bannister, G. H. Mitchell (GB), Ultrastructural changes in membranes during the asexual erythrocytic cycle of *Plasmodium knowlesi* and *P. falciparum* abstr. 17

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structure and cytochemistry of the myxozoan, *Chloromyxum trijugum* abstr. 219

J. F. Fahrni (Switzerland), Scanning microscopy and protargol staining of budding and metamorphosis in the sessile ciliate *Spirochona gemmipara* (*Ciliophora, Chonotrichida*) abstr. 92

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Chairman: Isabel Cunningham, University of Edinburgh, Edinburgh, GB
Co-Chairman: Louis Diamond, National Institutes of Health, Bethesda, USA
Convener: Witold Kasprzak, Academy of Medicine, Poznań, Poland

L. S. Diamond (USA), Axenic cultivation of *Entamoeba* and *Trichomonadidae*: the state of the art abstr. 74

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C. G. D. Brown (GB), *Theileria*

R. Brun (Switzerland), *In vitro* cultivation of *Trypanosoma (T.) brucei* abstr. 41

L. D. Hendricks (USA), Cultivation of *Leishmania in vitro*: promastigotes and amastigotes abstr. 135

I. Roitman (Brasil), *In vitro* cultivation of *Trypanosoma cruzi* abstr. 316

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L. Červa (Czechoslovakia), Differences in the growth requirements of virulent and nonvirulent strains of *Naegleria fowleri* abstr. 50

Symposium C: Cellular Membranes: Structure and Function

Chairman: Richard D. Allen, University of Hawaii at Manoa, Honolulu, USA
Co-Chairman: John E. Thompson, University of Waterloo, Waterloo, Canada
Convener: Aleksandra Przełęcka, Nencki Institute of Experimental Biology, Warsaw, Poland

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Y. Nozawa, S. Nagao (Japan), Regulation by calmodulin of membrane-bound guanylate cyclase in *Tetrahymena* abstr. 274
H. Plattner (FRG), Membrane fusion in ciliates and other protozoa, with special emphasis on exocytosis abstr. 294 and proc. II 267–278
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K. Hiwatashi, A. Kitamura (Japan), Ciliary membranes in the mating reactions of *Paramecium* abstr. 140

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A. Przełęcka, A. Sobota (Poland), Structural changes of plasma membrane in differentiating trophozoites of *Acanthamoeba castellanii* abstr. 297

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A. L. Knupfer, D. Coral, J. Gruenberg, J. Deshusses (Switzerland), Physiological effects of liposome-trypanosome interactions abstr. 189

C. F. Bardele (FRG), Freeze-fracture technique as a new tool in studying ciliate phylogeny abstr. 18

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Chairman: Miklos Müller, Rockefeller University, New York, USA
Co-Chairman: Jan Michejda, Mickiewicz University, Poznań, Poland
Convener: Maria Jerka-Dziadosz, Nencki Institute of Experimental Biology, Warsaw, Poland

M. Müller (USA), Introduction

J. Michejda (Poland), Protozoan mitochondria abstr. 249

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J. R. Nilsson (Denmark), The dynamics of cell organelles in *Tetrahymena pyriformis* abstr. 270

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A. Čerkasova, J. Čerkasov, J. Kulda (Czechoslovakia), Structure and function of hydrogenosomes; a review abstr. 48

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Chairman: Karl E. Wohlfarth-Bottermann, University of Bonn, Bonn, FRG
Co-Chairman: D. Lansing Taylor, Harvard University, Cambridge, USA
Convener: Andrzej Grębecki, Nencki Institute of Experimental Biology, Warsaw, Poland

K. E. Wohlfarth-Bottermann (FRG), Ultrastructure and molecular background of motility. Introduction proc. I 113–116

A. Grębecki (Poland), Supramolecular aspects of amoeboid movement proc. I 117–130

P. Satir (USA), Tubulin-based motility in *Protozoa* abstr. 327 and proc. I 131–140

M. A. Sleigh and D. I. Barlow (UK), The use of cilia by *Protozoa* abstr. 371 and proc. I 141–147

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T. Maruyama (Japan), Retractile motion of the longitudinal flagellum in a marine dinoflagellate, *Ceratium tripos* abstr. 236

C. Gicquad, M. Paulin-Levasseur (Canada), Electron microscopy of the contractile structures in cytoplasmic preparations of *Amoeba proteus* abstr. 113

L. Grębecka (Poland), Formation of new fronts between two contraction sites in *Amoeba proteus* abstr. 123

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J. Sikora (Poland), Organization of cytoplasmic streaming in *Paramecium* abstr. 337

B. Hrebenda (Poland), Ultrastructure of the frontal part of *Physarum polycephalum* abstr. 147

K. Pożaryska (Poland), E. Voigt (FRG), An unique mode of attachment of foraminifers to substratum abstr. 296

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Chairman: Anthony T. Soldo, Veterans Administration, Medical Center, Miami, USA
Co-Chairman: Klaus Heckmann, University of Münster, Münster, FRG
Convener: Witold Kasprzak, Academy of Medicine, Poznań, Poland

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D. S. Weis (USA), The process of infection of aposymbiotic *Paramecium bursaria* by algae abstr. 388
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Chairman: David L. Nanney, University of Illinois, Urbana, USA
Co-Chairmen: Gerard de Haller, Universite de Geneve, Geneve, Switzerland
Joseph Frankel, University of Iowa, Iowa City, USA
Renzo Nobili, Istituto di Zoologia, Pisa, Italy
Convener: Maria Jerka-Dziadosz, Nencki Institute of Experimental Biology, Warsaw, Poland

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Tchang Tso-run, Dong Ji-long, Gu Fu-kang, Huang Ming (China), Experimental study of macronucleation and macronuclei displacement on artificial dorsi-conjugant *Stylonychia mytilus* and *Oxytricha fallax* abstr. 361

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M. Fujishima (FRG), Pre-meiotic DNA synthesis in *Paramecium caudatum* abstr. 106

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Chairman: Juri Teras, Estonian Academy of Sciences, Tallinn, USSR
Co-Chairman: Richard Adekundle Joshua, Nigerian Institute for Trypanosomiasis, Vom Plateau, Nigeria
Convener: Tadeusz Dzbeński, National Institute of Hygiene, Warsaw, Poland

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R. K. G. Assoku (Ghana), Studies in African trypanosomiasis: the identification and nature of the B-cell mitogen of *Trypanosoma congolense* abstr. 15

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Chairman: Lucilla Arcay, Universidad Central de Venezuela, Caracas, Venezuela
Co-Chairman: Pilar Alonso, Instituto de Immunologia y Biologia, Microbiana, Madrid, Spain
Convener: Andrzej Grębęcki, Nencki Institute of Experimental Biology, Warsaw, Poland

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In Memory of Professor Theodore L. Jahn

Chairman: Cecily Chapman-Andresen, University of Copenhagen, Copenhagen, Denmark
Convener: Stanisław Dryl, Nencki Institute of Experimental Biology, Warsaw, Poland

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Chairman: Gotram Uhlig, Helgoland Meeresstation, Helgoland, FRG
Convener: Witold Kasprzak, Academy of Medicine, Poznań, Poland

W. Foissner (Austria), Terrestrial Protozoa as bioindicators: investigations at a graded ski trail at the Schlossalm near Bad Hofgastein abstr. 96

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W. Schönborn (GDR), Annual production and population dynamics of *Testacea* in soils abstr. 329

M. M. Coûteaux (France), Relations trophiquez chez les Protozaires du sol abstr. 64

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N. Willumsen (Denmark), Marine amoebae (*Gymnamoebia*) cultured from benthic habits of the Danish Belt Sea abstr. 392

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M. R. Goddard (GB), Temporal variations in the population dynamics of ciliate protozoa in slow sand filter beds abstr. 115

L. Bowik-Dylińska (Poland), Phosphorus and nitrogen excretion rate by free living planctonic ciliates abstr. 38

J.F. McClellan, J. Frey, M. K. Campion, D. C. Coleman (USA), Protozoan mineralization roles in soil ecosystems abstr. 241

H. F. Larsen (Denmark), Ecological and light microscopic studies of *Blepharisma lateritium* Kahl, 1932 (*Trichoda ignita* O. F. Müller, 1778) abstr. 212

K. Schlott-Idl (Austria), The ecology of some pelagic ciliates of Piburger See (Tyrol, Austria) abstr. 328

M. Cigada Leonardi (Italy), Speciation in fresh water amoebae abstr. 57

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Chairman: Warren Jonas, Wallaceville Animal Research Center, Upper Hutt, New Zealand

Co-Chairman: Jan Kučera, Czechoslovak Academy of Sciences, Prague, Czechoslovakia
Convener: Witold Kasprzak, Academy of Medicine, Poznań, Poland

S. Urdaneta-Morales (Venezuela), *In vitro* interaction of *Trypanosoma cruzi* with poikilotherm and homoiotherm cells abstr. 376

F. A. Ghaffar, E. Scholtyseck (FRG), Ultrastructural studies on the host-parasite relationships and pathological changes induced by *Eimeria falciformis* (*Coccidia, Sporozoa*) infection abstr. 410

E. U. Canning (GB), The parasite/host cell interface in microsporidian infections abstr. 47

T. V. Beyer, J. S. Grikenene, N. V. Sidorenko (USSR), *Sarcocystis ovifelis* (*Eimeriidae, Sporozoa, Apicomplexa*): modes of asexual reproduction in the cyst stages abstr. 27

W. Kasprzak, A. Majewska (Poland), Relationship between *in vitro* excystation and invasiveness of *Giardia cysts* abstr. 175

E. Roigas, J. Teras, I. Kazakova, H. Sardis (USSR), On the role of *Trichomonas tenax* in genesis of pulmonary inflammations abstr. 315

M. R. Hidalgo-Arguello, M. Cordero del Campillo (Spain), Kinetics of the elimination of oocysts of *Eimeria* spp. in ovines in rough pasture abstr. 137

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K. Vickerman, L. Tetley (GB), Differentiation of the metacyclic stage in the life cycle of *Trypanosoma brucei* abstr. 381

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L. Dyková, J. Lom (Czechoslovakia), G. Grupcheva (Bulgaria), Fish gills at the site of sporogony of an *Eimeria* species abstr. 85

K. Migała (Poland), From researches on parasitic ciliata on salmonids in pond culture abstr. 250

K. Purrini (FRG), Protozoa of soil fauna in beech forests, mixed coniferous forests and mixed deciduous forests abstr. 298

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Z. J. Stelmaszyk (Poland), Lambliasis in young men's population abstr. 378

R. L. Beaudoin, N. D. Pacheco, R. J. Moon, R. A. Vrable, R. D. Leunck (USA), Establishment of *Plasmodium berghei* in mice with special reference to the liver abstr. 23

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A. Marot (Yugoslavien), Die Möglichkeit für die Diagnostizierung der Sarkosporidien in den Muskelfasern, ohne dass die Mischerschen Schläuche sichtbar Sind abstr. 233

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Z. Hostounsky (Czechoslovakia), A method prolonging the period of release of microsporidian spores with the faeces abstr. 146

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Chairman: Miklos Müller, Rockefeller University, New York, USA
Co-Chairman: Jan Michejda, Mickiewicz University, Poznań, Poland
Convener: Andrzej Grębecki, Nencki Institute of Experimental Biology, Warsaw, Poland

C. Chapman-Andresen (Denmark), Peroxidase activity in large freshwater amoebae abstr. 53

N. Yarlett, D. Lloyd, A. G. Williams (Scotland), Hydrogenosomes of the rumen protozoon *Dasytricha ruminatium* Schuberg abstr. 397

P. M. Paeste, A. K. Fok, R. D. Allen (USA), Lysosomal enzymes of *Paramecium* abstr. 284

A. Tiedtke (FRG), Secretion of hexosaminidase and acid phosphatase associated with mucocyst discharge in *Tetrahymena thermophila* abstr. 367

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D. Fischer-Defoy (FRG), Ultrastructural and cytochemical studies of the oral apparatus and digestion in the ciliate *Climacostomum virens* abstr. 95

K. Hausmann (FRG), D. J. Petterson (GB), The feeding activity of the heliozoon, *Actinophrys sol* abstr. 133

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M. Freiburg, S. Rommel (FRG), Effect of cycloheximide on the activity of RNA polymerase I in the macronucleus of *Tetrahymena pyriformis* abstr. 101

G. Steinbrück (FRG), Age-dependent amplification of individual genes in the macronucleus genome of *Stylonychia mytilus* abstr. 347

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B. Skoczylas (Poland), Development of *Paramecium aurelia* grown in the presence of 4,6-diamidine-2-phenylindole (DAPI) — a DNA ligand abstr. 340

W. Krawczyńska (Poland), Prolongation of *Tetrahymena* cilia regeneration under the influence of Diamidine Phenylindole (DAPI) abstr. 197

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International Collaboration Among Protozoologists During the Years 1961 to 1981 [1]

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The Sixth International Congress of Protozoology in Warsaw (5–11 July, 1981) marked the end of the first 20 years of organized collaboration of world protozoologists. This anniversary appeared to be a suitable occasion for analysis and evaluation of not only the evolution of the Congresses and of the forms of international collaboration, but also of the subject matter under investigation.

1. International Organizations of Protozoologists

1.1. Society of Protozoologists

International collaboration of protozoologists started only after the second world war. The initiative in this area was undertaken by American scientists. In 1947 there was organized the American Society of Protozoologists which, from that time, started to hold annual meetings in the United States. From its inception, the Society, despite its name, included in its ranks investigators from other countries. At its Third Annual Meeting, in 1951, the members of the Society adopted the first Constitution and By-Laws. In its desire to assume and proclaim its international, rather than national, character, the Society changed its name from American Society of Protozoologists to Society of Protozoologists. This change appeared on the cover of Volume 3 of the Proceedings of the Society's Fourth Annual Meeting. Until 1954, these Proceedings constituted the only publications of the Society. However, in February,

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This article was prepared for Post-Congress Volume as a special contribution to celebration of twentieth anniversary of Congresses.

[1] Reprint requests may be sent to either of the authors at their respective addresses.

1954, Vol. 1, No. 1 of the "Journal of Protozoology" appeared. In that number, Professor D. H. Wenrich, in his President's Address, reviewed the history of the young Society. From its very beginning, the Journal assumed an international character by including on its Editorial Board members residing outside the United States (Profs. Fauré-Fremiet from France, and Pringsheim from the Federal Republic of Germany), and by announcing its readiness to publish in French and German, in addition to English; in fact, one of the contributions to the first issue was written in French by Professor Fauré-Fremiet. The traditions of publishing in languages other than English and having protozoologists from outside the United States as members of the Editorial Board (more recently called Board of Reviewers), have been preserved to date.

In 1961, during the First International Congress of Protozoology, in Prague, the Society had 600 members. The organizers of the Congress proposed originally that the First Congress would also be the Thirteenth Annual Meeting of The Society of Protozoologists. Subsequently, however, this idea was abandoned. There was, however, the precedent established that during this and future Congresses of Protozoology, there would take place the Business Meeting and the Executive Committee meeting of The Society of Protozoologists; thereafter, starting with the Third Congress, the Past President's Address of the Society were delivered during the Congresses. The international character of the Society was further emphasized in the 1960's, when the British and Israeli Sections were organized.

In the middle of the 1960's the protozoological organization under the name "Groupement des Protozoologues de la Langue Français" was formed, which questioned the status of The Society of Protozoologists as an organization representing all protozoologists. During the Second International Congress of Protozoology in London, 1965, the representatives of the "Groupement" proposed formation of the International Union of Protozoological Societies, in which The Society of Protozoologists would represent only one national organization; however, the proposal was never implemented. On the other hand, there was formed, in the course of the London Congress the International Commission on Protozoology, which was to establish connections with international organizations such as IUBS, UNESCO, WHO, and FAO, and actively to participate in the preparation of the subsequent International Congresses of Protozoology. Since the London Congress, more national or supranational protozoological societies were formed. Still, The Society of Protozoologists, which retained its international status, prospered; in addition to the already existing British and Israeli Section there were formed within its framework also Scandinavian, Italian, Brazilian, Argentinian, and German (Federal Republic of Germany) Sections.

During the Fifth International Congress in New York, in 1977, it was resolved that various groups of protozoologists could affiliate themselves with The Society of Protozoologists as sections or as national or supranational organizations. Such affiliated organizations, when prepared (and able) to cover the cost of publication, could have summaries of their national meetings printed in the supplement to "The Journal of Protozoology" without the individual members of the organizations having to pay dues to the Society. The Section of Protozoologists of the Polish Zoological Society is one of these national organizations, affiliated with The Society of Protozoologists.

Following tradition, during the Sixth International Congress in Warsaw, 10 July, 1981, there took place in the Hotel Europejski an Annual Business Meeting of The Society of Protozoologists, with the President, the late Professor R. Barclay McGhee, in the chair. His short presentation was very optimistic about the future of protozoology and protozoologists. He mentioned also the formation of The Society fo Protozoologists of the People's Republic of China.

The Society of Protozoologists is growing in number of members as well as in the establishment of new sections. In June of 1981 the Society itself had a total of 996 members; during the next year, 82 new members joined. Continued interest in the field is reflected in the still significant number of requests for the little booklet published by the Society under the title "So You Want To Be a Protozoologist?"

The optimism expressed by President McGhee was confirmed by the reports of the Secretary (Professor Jerome J. Paulin) and Treasurer (Professor Brower R. Burchill). In the period between 30 June 1980 and 31 May 1981, there was a significant improvement in the Society's finances; the excess of income over expenditures increased from $ 18,000 to over $ 32,000.

1.2. International Commission on Protozoology

On 5 September 1965, during the closing session of the Second International Congress of Protozoology in London, the International Commission on Protozoology was established. Both its structure and scope of activities were defined. It was resolved that the Commission would consist of delegates of national and/or supranational Sections or Societies of Protozoologists; the President and Secretary-General of the previous Congress; and the President and Secretary-General of the next Congress, who were to be simultaneously President and Secretary of the Commission for the interim four-year period. It was decided also that the Third International Congress of Protozoology was to be held in Leningrad in 1969.

Starting with the London Congress, the International Commission became the most important international organization of Protozoologists. The Commission selects the places and approves the programs of the Congresses and the slate of invited guests.

During the Third International Congress of Protozoology in Leningrad (1969), the International Commission formalized its relationship with IUBS and established the rules governing the constitution of its membership. According to these rules, national or supranational societies or sections consisting of fewer than 100 members have one representative each; two representatives are allowed such units that have 100–300 members; and units with more than 300 members have three representatives. During the periods between Congresses, the International Commission promotes the development of protozoology and facilitates personal contacts among all protozoologists.

During the 1970's the International Commission undertook a series of steps aimed at improving the research methods and increasing the quantity and improving the quality of publications. The Commission resolved to meet once every two years. The meetings between Congresses are to be used for discussion and ultimate choice of the scientific subjects to be discussed at the next Congress; the members are also expected to concern themselves with certain organizational details of such Congresses. Starting with the Fourth International Congress of Protozoology, 1973, in Clermont-Ferrand, beyond publishing summaries of contributed papers, the organizers have been asked to publish volumes containing the various special lectures and summaries of the deliberations of round-table discussions and of symposia.

At the Fifth International Congress in New York (1977), further organizational changes in the Commission were introduced. In an attempt to improve international coordination during the periods between Congresses, it was resolved that the functions of the coordinator would be fulfilled during the succeeding four years by the Secretary-General of the previous Congress. His primary tasks would include distribution of information about national protozoological meetings and symposia, and about new publications.

The general program format of the Fifth International Congress in New York was adopted by the Commission meeting in Warsaw in 1979, although with some important modifications, for the Sixth International Congress. The most important modification was the change of workshops to real Symposia, and a clear separation of the latter from contributed paper sessions. In addition, a major round-table discussion on "Phylogenetic Relationships Among Protozoa" was scheduled for the entire morning of the last day of the meetings. The foremost experts in the field participated in the discussion, which is printed in this volume.

It is hoped that similar discussions on other topics will be held at future Congresses. The most recent meeting of the International Commission took place during the Sixth Congress, July 1981, in Warsaw. There was much discussion about the site of the Seventh Congress, which is scheduled for 1985. Ultimately, two countries presented formal invitations to the Commission — Japan (through Professor Inoki) and Kenya (through Dr. Mutinga). As a result of a vote, Kenya was selected as the site of the Seventh Congress. Dr. Mutinga expressed his thanks to the members of the Commission. The selection of a developing country, outside Europe and North America, is a sign of a new period of growth in protozoology.

2. The Congresses of Protozoology

An especially effective form of international collaboration in studies of protozoa is represented by the International Congresses. Between 1959 and 1960 Czech, Soviet, and Polish protozoologists initiated attempts to organize European meetings. This was the seed for the First International Congress in Prague, the organization of which was undertaken by Professor Jírovec with his closest collaborators (J. Ludvík, J. Lom, J. Vavrá, and J. Weiser). In the course of preparing for the Congress, The Society of Protozoologists, acting through Professors J. O. Corliss, N. D. Levine, and W. Trager, offered its help in the organization of the meetings. The Organizing Committee also included two Soviet investigators, G. I. Poljansky and E. M. Kheissin.

The First Congress met for 10 days (22–31 August, 1961). A total of 260 (including accompanying persons) participated, representing 23 countries. The most numerous were the groups from the United States and Czechoslovakia. The sessions of the Congress (the official name was The First International Conference of Protozoology) were all plenary (with the exception of one on *Toxoplasma*) and all of them took place in the International Hotel. The subjects considered by the Congress were divided into the following groups: systematics; genetics; biochemistry; biophysics; cytology; ecology; electron microscopy; parasitic protozoa; and *Toxoplasma*. There also were two sessions during which scientific films were shown. Three lectures had a more general character.

The symbol of the conference was *Giardia lamblia* (*Lamblia intestinalis*), a parasitic flagellate found in the small intestine of man which was discovered in 1895 by a Czech physician, Wilem D. Lambl; it has remained the symbol of all subsequent Congresses. The Second International Conference on Protozoology took place in London between

25 July and 5 August, in 1965. It included about 450 participants. Plenary sessions were held in the mornings and in the afternoons simultaneous contributed paper sessions. The main responsibility for organizing and running the meetings rested upon the shoulders of the President, Professor P. C. C. Garnham and the Secretary-General, Dr. R. S. Bray. The opening sessions consisted of six lectures. The following days were divided into 15 sessions, plus one for scientific films.

During the Third International Congress of Protozoology, 2–10 July, 1969, in Leningrad, over 300 protozoologists from the Soviet Union took part, which brought the total number of participants to 700. The President of the Congress was Professor G. I. Poljansky, and the Secretary-General was Dr. I. B. Raikov. The format did not differ significantly from that of the Second Congress in London. In Leningrad the topics under discussion were dominated by parasitology, especially in the area of ecology and adaptation of parasitic protozoa. There were fewer contributions on morphogenesis and life cycles, but a significant number in the area of morphology, especially the ultrastructure of ciliates. The sections devoted to biochemistry and physiology were rather heterogeneous in nature. As in Prague and London, there were separate film sessions.

The Fourth Congress, which took place in Clermont-Ferrand, France, included about 570 participants. Major changes occurred in the structure of this gathering, in comparison with the preceding ones. In addition to plenary and contributed paper sessions, there were 26 Round-Table Discussions (or workshops). Each discussion dealt with a rather narrow topic; the chairmen and vice-chairmen of each were asked to present summaries of the deliberations. The following topics were taken up during the round-table discussions: purine and pyrimidine metabolism; extranuclear DNA; extranuclear inheritance; regulation of morphogenesis; conjugation and genetics of free-living protozoa; nuclei of ciliates; nuclear division; and stomatogenesis. In addition, several sessions dealt with the phenomena of movement, including ameboid, ciliary, and flagellar movements, and the processes of contractions, as well as with the developmental cycles of protozoa, their ecology, and species found in salt water, fresh water, and in the soil. Questions of parasitism were also discussed.

The Fifth Congress, held in New York in 1977, attracted a large number of participants, the total number, including accompanying persons, being 900. In addition to plenary session lectures, symposia, round-table discussions, and contributed paper sessions, the poster session was introduced. In the course of the Congress, two specialized symposia took place, one on Paleoprotistology and the second on Chagas Disease. As be-

fore, parasitology was well represented, with emphasis upon the problems of parasitism and symbiosis — about 50% of all contributions. According to tradition, strong emphasis was also placed upon morphogenesis and morphology; taxonomy; and evolution. The biochemical and physiological sections had, like those in the preceding Congress, a rather heterogeneous character. Among them was a round-table discussion of the behavioral, physiological, and molecular aspects of movement.

The Sixth Congress, held in Warsaw in 1981, was not as well attended as the previous ones, with 392 participants from 34 countries. On the basis of preregistration, the organizers had anticipated about 500. Nonetheless, and despite numerous difficulties, the meeting in Warsaw was a great success, characterized by a very friendly atmosphere. The experience gained from the previous meetings or Congresses was utilized, and there was an attempt to balance several types of presentation for the exchange of information. Of special importance during the deliberations in Warsaw was the round-table discussion in which panel members attempted to reconcile numerous questions in the realm of systematics and phylogeny of protozoa.

In analyzing the past Congresses, it is easy to note the changes of direction in protozoology which occurred between 1961 and 1981. Certain subjects predominated during the first two Congresses, to which less time was given in the later ones, reflecting these changes. From the Prague Congress to the Warsaw Congress, there was a steady improvement in organization, continually aiming at more suitable and varied forms of presentation. The Congresses of Protozoology never became gigantic, and this renders them especially conducive to preserving old and establishing new contacts among protozoologists, providing a very successful forum for the free exchange of ideas.

The accompanying Table 1 contains the numbers of authors and the numbers of their summaries or abstracts that were printed in the Pro-

Table 1

The numbers of abstracts and the numbers of authors of the abstracts published in the Proceedings ("Progress in Protozoology") of the several Congresses held to date

International Congress of Protozoology	No. of abstracts	No. of authors
I Prague (1961)	192	230
II London (1965)	374	467
III Leningrad (1969)	492	623
IV Clermont-Ferrand (1973)	479	525
V New York (1977)	478	745
VI Warsaw (1981)	418	866

ceedings (published as "Progress in Protozoology") of the several Congresses. This listing reveals the continual drop in the number of single-authored publications; co-authorship has become the general rule, a development which highlights the need for collaboration among various specialists.

3. International Periodicals and Publications Connected with the Congresses

Protozoa are utilized as model organisms in basic investigations in the areas of biochemistry, physiology, genetics, ecology, among others. A significant number of important pathogenic species renders them also important for human and veterinary medicine. In light of the foregoing, reports of investigations on protozoa appear in a variety of periodicals, while the number of specialized international journals devoted exclusively to this group of organisms has remained rather small.

At the time of the First International Congress (Conference) of Protozoology in Prague, there were only two international periodicals devoted to protozoa: "Archiv für Protistenkunde" (published since 1902) and "The Journal of Protozoology", established in 1954 under the auspices of The Society of Protozoologists. The increased activity of protozoological research, stimulated in part by the International Congresses, resulted in the establishment of two additional international periodicals — "Acta Protozoologica" (published in Warsaw since 1963) and "Protistologica" (published since 1965 in Paris by the Centre National de la Recherche Scientifique).

During the past 20 years, an important role in exchange of information has been played by the publications connected with the International Congresses. Two years after the Congress in 1961, the Czechoslovak Academy of Sciences published a volume entitled "Progress in Protozoology", Proceedings of the First International Congress, held in Prague, which included all the contributions presented at the Congress. The British, following the precedent of the Czechs, published a booklet under the same title, which was distributed at registration. This booklet contained summaries of the communications submitted for presentation at the Congress. The publication prepared for the London Congress fulfilled two functions: it served as proceedings and also as a post-Congress publication. The two publications prepared before the Leningrad Congress (one version in Russian and the other in the three other official languages of the Congress, English, French, and German) contained summaries of all the contributions. Both versions were entitled "Progress in Protozoology" ("Uspiechy Protozoologii," in Russian).

Further modifications in the area of publications were introduced at the Fourth Congress, held at Clermont-Ferrand. The participants received a volume at registration containing summaries of the plenary lectures and contributed papers. This volume, prepared by photo-offset, carried the traditional title of "Progress in Protozoology". In addition, the organizers of the Congress published, in 1974, "Actualités Protozoologiques," containing the summaries prepared by the chairmen of individual round-table discussions. The Fifth Congress, which was held in New York in 1977, followed the French pattern, i.e., pre- and post-Congress publications were offered to the participants.

In Warsaw, participants in the Sixth Congress were given a volume ("Progress in Protozoology") containing summaries of the various presentations. Two volumes of post-Congress publications were also planned. The first one appeared at the end of 1982, and the second is to appear during 1984.

4. Final Remarks

Protozoology, which can be traced from the work of Anthony van Leeuwenhoek, did not become a separate discipline of biology before the latter part of the 19th century, but it became the subject of international collaboration much later. This collaboration started on a large scale only after the second world war, and it assumed a more organized form during the last 20 years. From this viewpoint, one can observe some analogy between biophysics and protozoology. The former also came into its own in the 19th century, and the first Congress devoted to it took place in 1961. There are, however, real differences between the two disciplines. These pertain not only to the number of investigators, but also to their very structure. Protozoology is a science whose unifying factors are the organisms investigated (i.e., protozoa) while in biophysics the unifying factors lie in methodology. It is evident that either group of factors is strong enough to stimulate the development of the two disparate disciplines.

A critical analysis of developments in protozoology during the past 20 years reveals a continuous change in direction of this discipline. Also evident is the steady improvement of international collaboration among world protozoologists, as fostered by the quadrennial International Congresses and by the activities of the International Commission on Protozoology.

EXPLANATION OF PHOTOGRAPHS

I International Congress of Protozoology
Prague, 1961

Phot. 1. Participants of the Congress before the Hotel International (photo, Progress in Protozoology, Prague 1961)
Phot. 2. During session at the Hotel International (photo, J. Lom)
Phot. 3. E. M. Cheissin and R. Yagiu (photo, S. L. Kazubski)
Phot. 4. W. Balamuth, D. R. Pitelka and W. A. Siddiqui (photo, S. L. Kazubski)

II International Congress of Protozoology
London, 1965

Phot. 5. J. O. Corliss, J. Dobrzańska-Kaczanowska and E. Fauré-Fremiet
Phot. 6. A. Okajima, T. H. Abe and R. Yagiu (photo, S. L. Kazubski)

III International Congress of Protozoology
Leningrad, 1969

Phot. 7. The group of participants of the Congress (photo, organizers of the Congress)
Phot. 8. The opening session of the Congress (photo, organizers of the Congress)
Phot. 9. M. A. Peshkov and R. D. Allen (photo, S. L. Kazubski)
Phot. 10. At the coffee break (photo, S. L. Kazubski)

IV International Congress of Protozoology
Clermont-Ferrand, 1973

Phot. 11. J. Sikora before the Department of the Medicine and Pharmacy, University of Clermont (photo, L. Kuźnicki)
Phot. 12. The main entry to the Congress (photo, J. Sikora)
Phot. 13. Participants of the Congress on the way to the group photography (photo, S. L. Kazubski)
Phot. 14. T. M. Sonneborn (photo, S. L. Kazubski)
Phot. 15. T. L. Jahn and K. E. Wohlfarth-Bottermann (standing back to the camera)

V International Congress of Protozoology
New York, 1977

Phot. 16. Participants of the Congress before the Fordham University at Lincoln Center (photo, organizers of the Congress)

VI International Congress of Protozoology
Warsaw, 1981

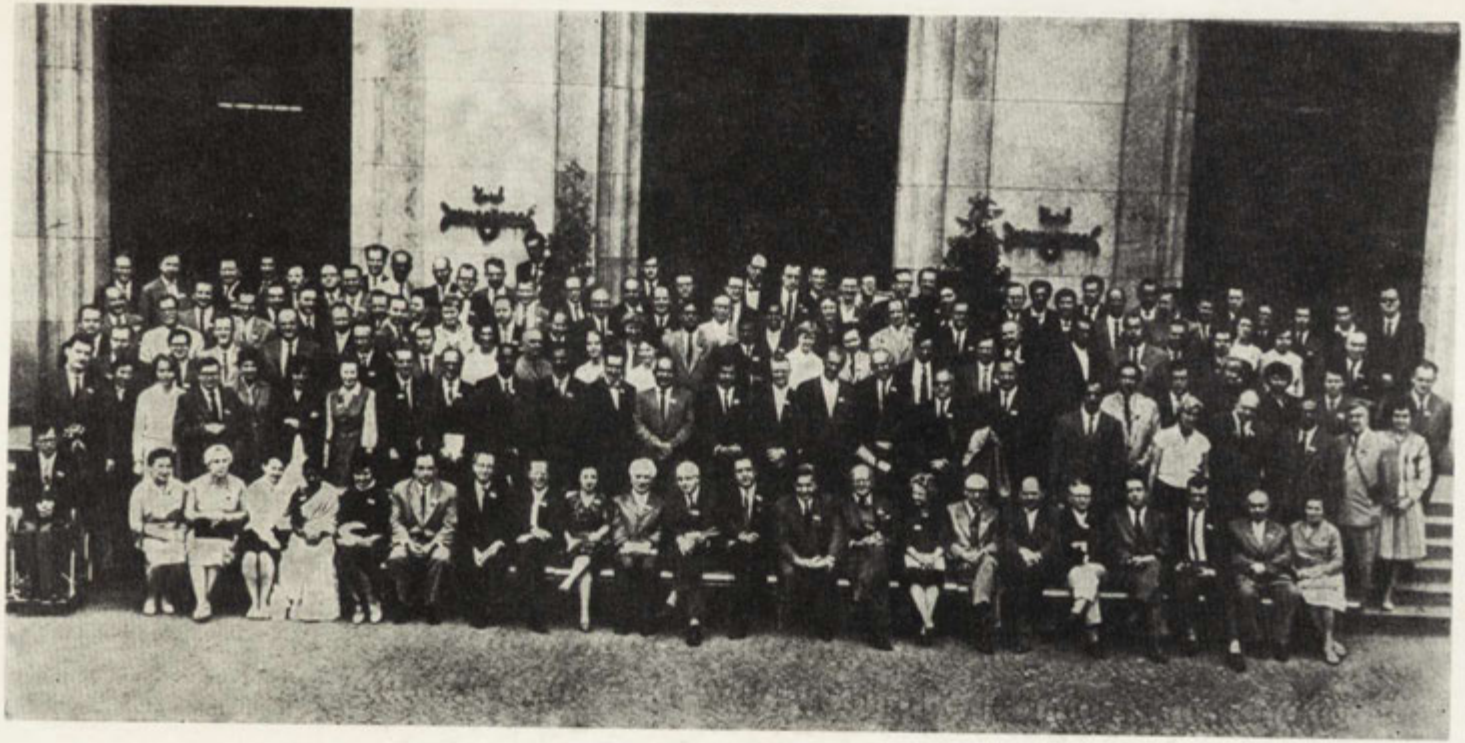
Phot. 17. The opening session of the Congress. From the left: S. Dryl, M. Jerka-Dziadosz, S. L. Kazubski, J. Lom, B. M. Honigberg, J. H. Teras, A. Gieysztor, J. Płoszaj, A. Urbanek, L. Kuźnicki, R. Nobili, R. B. McGhee, J. Jadin, W. Kasprzak, P. C. C. Garnham, E. Vivier, J. I. Poljansky, J. Lee, J. R. Nilsson, S. Inoki and W. Trager (photo, K. Krawczyk)

Phot. 18. Participants of the Congress in Concert Hall at the Fryderyk Chopin Academy of Music (photo, S. Bałuk)

Phot. 19. Meeting and banquet of the Protozoological Society at the Europejski Hotel. Standing: P. C. C. Garnham, R. B. McGhee, J. I. Poljansky, sitting: R. D. Allen, Mrs. Allen, S. L. Kazubski, J. Mann and J. Wolfe (photo, S. Bałuk)

Phot. 20. W. Trager's toast to the success of the Congress and further collaboration among protozoologists. To the left: Mrs. Trager, I. Filipowicz, to the right: E. Wyroba and R. Nobili (photo, S. Bałuk)

PRAGUE 1961



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CLERMONT-FERRAND 1973

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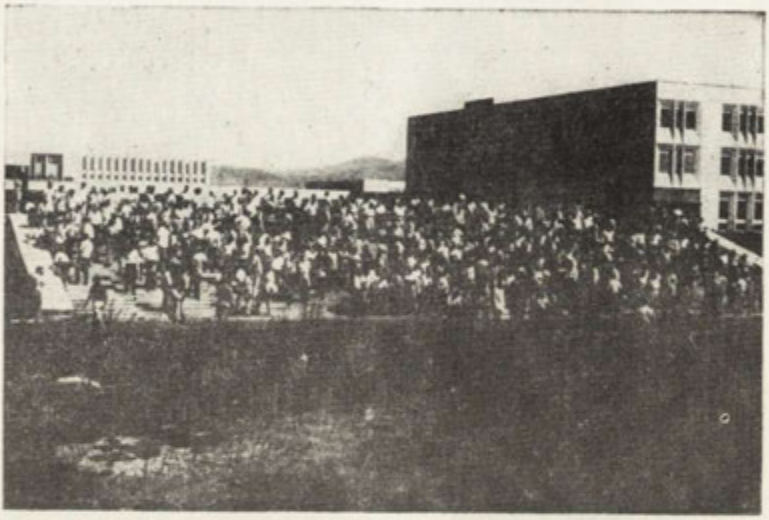
CLERMONT-FERRAND 1973



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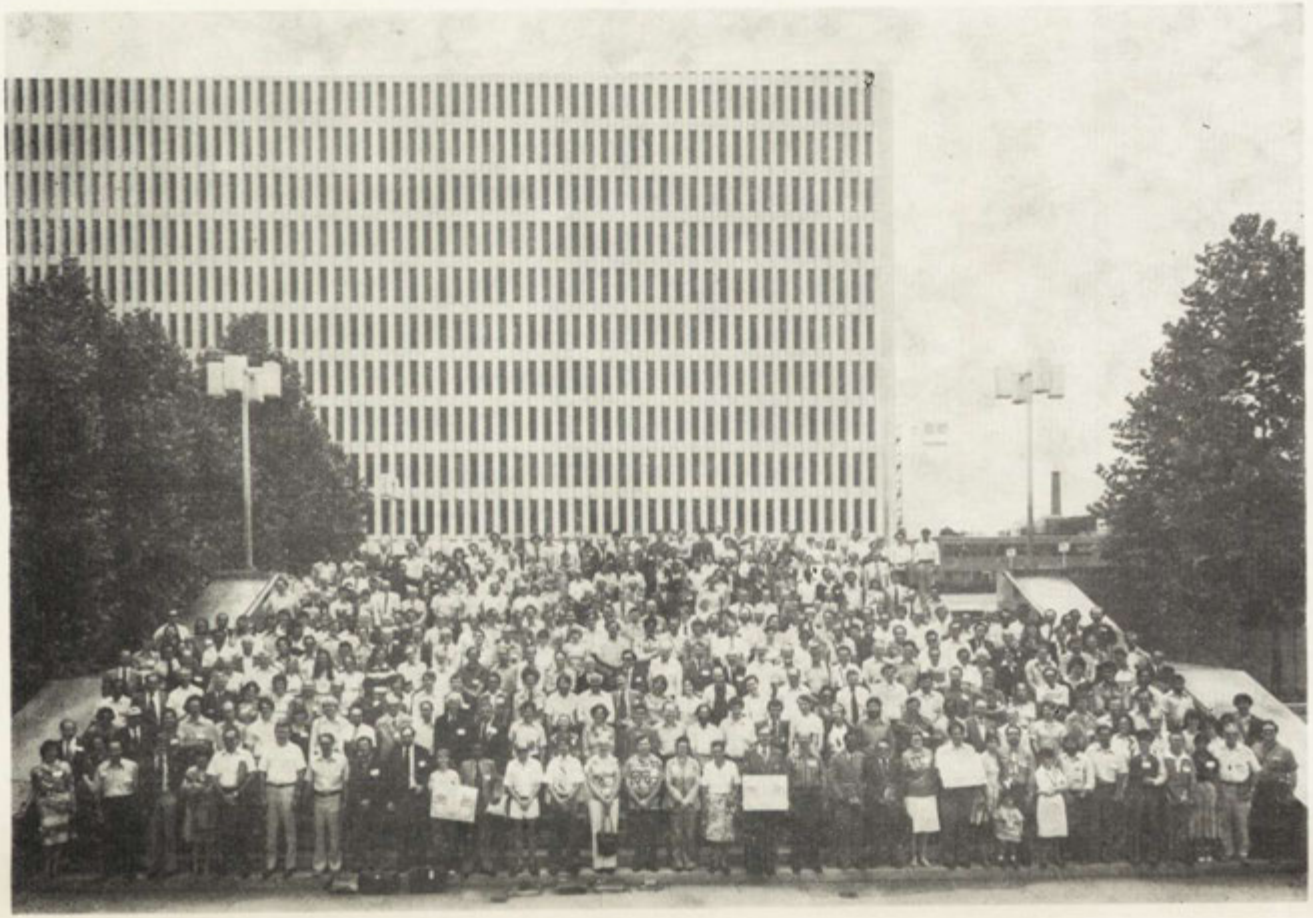
NEW YORK 1977



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