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# Polarity of Motor Function in *Amoeba proteus* II. Non-locomotory Movements

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*Synopsis.* The locomotion cannot be considered as only manifestation of the motor activity of amoeba. Such phenomena as cytokinesis, capping, pinocytosis and phagocytosis belong to the same category, because the movement is at least a necessary component of their manifestation and because the intracellular contractile apparatus is involved.

Cytokinesis in *Amoeba proteus* depends probably on the accumulation of actin and myosin filaments in the form of a contractile ring at the equatorial plane; its contraction results in cell fission, the contractile material of the ring probably gives origin to the uroids of both daughter amoebae, and thus it determines their future motor polarity.

Capping in amoebae is less explored than in other motile cells; in particular the capping of lectins by *A. proteus* needs more investigation. On the other hand, some other soluble ligands and particulate surface markers are known to be transported backwards and accumulated in the form of caps on the surface of the posterior pole of moving amoeba.

The same polar localization characterizes the permanent pinocytosis in *A. proteus*. This type of pinocytosis occurs spontaneously without interrupting the cell movement. Its specifically posterior localization may be related to the ionic conditions in the uroidal region, the strong folding of the cell surface in the same area and, perhaps, a spontaneous capping of some receptors which are continuously recycled between the cell surface and interior.

The motor system of amoeba is still more engaged in the phenomena of induced pinocytosis and phagocytosis (formation of specific pinocytotic or phagocytotic pseudopodia, followed by the cessation of locomotion). The phagocytosis modifies the former cell polarity because it is orientated by the site of contact with the prey. In contrast

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to this, the induced pinocytosis is provoked by uniform stimulation; nevertheless, it begins always at the uroid, next it appears in the retracting pseudopodia, and finally becomes evenly manifested around the whole cell. The polar development of the induced pinocytosis is probably related to other manifestations of cell polarity: orientation of the endoplasmic streaming, differentiated surface folding degree, presence of the permanent pinocytotic channels at the uroid, which on their turn are all depending on the locomotion.

In general, the locomotion creates and maintains the cell polarity which in further consequence controls the manifestation of all other motor functions of amoeba.

Contractility of the submembraneous layer of microfilaments in *Amoeba proteus* is not only needed for cell locomotion. The same cortical activity is, moreover, engaged in producing some other kinds of intracellular movements, as capping, pinocytosis, phagocytosis and cytokinesis. The phagocytosis seems to be, like locomotion, a reaction orientated in space relative to the external stimulus acting locally on a part of the cell surface. Both these types of movements may be therefore involved in creating or modifying the cell polarity by the environment. The capping and pinocytosis on the contrary, may be both provoked by soluble inducers evenly distributed in the surrounding medium, i.e., by the stimuli uniformly attacking the whole cell surface. Therefore, the manifestation of the two latter phenomena is expected to depend on the pre-existing polarity of the cell.

The cytokinesis is generally recognized as a typical and important example of cell motility in eukaryotic cells (e.g., Mabuchi 1986), but in contrast to other movements mentioned above, it is preceded, accompanied and followed by the profound complex reorganization resulting in the division of the intracellular material. As a consequence the cytokinesis determines the future innate polarization of the structure and motor functions of daughter cells. Therefore, the present review of the mutual relations between the cell polarity and non-locomotory movements of amoebae, will begin with the cytokinesis.

## Cytokinesis in *Amoeba proteus*

The literature concerning the mechanism of mitosis in *A. proteus* is rather scarce. The first descriptions are due to Carter (1913), followed by Chalkley and Daniel (1933) and Dawson et al. (1937)]. Then, almost 50 years later, the mitosis of this species of amoeba has been thoroughly studied in the electron microscopy by Gromov (1985)

and, using monoclonal antibodies and colcemid, by Lorch and Jeon (1986).

Equally modest was the interest in the cell division of *Amoeba proteus*. The cytokinesis was described by Johnson (1930), Chalkley (1934) and (1951), Lewis (1942), and its role in the organization of the daughter cells was discussed by Goldacre and Lorch (1950). Recently, a new experimental study of this process was carried out by Rappaport and Rappaport (1986). All these descriptions of the morphology of cell division in *A. proteus* stress the numerous analogies with the cytokinesis of tissue cells. In amoeba, as in other cells, the mitotic apparatus determines the origin and the position of the cytokinetic furrow. The fission and separation of the daughter cells are result of tightening the contractile ring developed around the equator of the spherical dividing amoeba (Lewis 1942). The later idea of Chalkley (1935) that fission is provoked by the onset of locomotory activities in the two halves of the dividing amoeba, was recently discarded in profit of the contractile ring concept (Schroeder 1975), by the experiments of Rappaport and Rappaport (1986). It should be noted that all information concerning the presence and function of the contractile ring in the dividing amoebae were provided only by the study of living cells. Therefore, we must rely on other material and other sources, as far as the fine structure and molecular composition of the contractile ring is concerned.

The fine structure of the contractile ring was for the first time demonstrated in the electron microscopy by Schroeder (1968) in jelly-fish eggs. The actin microfilaments were found there to run parallel to the fission plane. Similar pattern has been described by other authors in various dividing tissue cells (see Mabuchi 1986) and in *Protozoa*, for example in *Tetrahymena* (Yasuda et al. 1980, Jerka-Dziadosz 1981) although Cohen et al. (1984) found in contractile ring of *Paramecium* a "granulo-fibrillar belt" instead the fibrillar layer. Also Grain (1986) postulates that in various Protista, the new category of cytoskeletal elements, non-actin filaments, are present. Probably the cytoskeletal components ensuring the motility or contractility processes are much more differentiated among protozoa than in metazoan cells.

The myosin was observed aggregated within the division furrow of the dividing tissue cells and is thought to participate in the structure of their contractile rings (Fujiwara and Pollard 1976, Herman and Pollard 1978). The involving of α actinin in the formation of the contractile ring and cleavage furrow was demonstrated by Mabuchi et al. (1985).

Microfilaments forming the contractile ring in tissue cells are certainly depolymerized after cell division and the whole ring structure is decomposed. It is not yet clear where and when the microfilaments are polymerized during arisal of the contractile ring. The F-actin filaments may arise by polymerization of G-actin in the area of their future activity, that is in the equatorial plane. Alternatively, it is possible that the contractile ring arises by retraction of ready actin polymers from the peripheral cell cortex and their aggregation around the equator. The continuity of the contractile ring and the cell cortex is well known in many tissue cells (see Oliver and Berlin 1982, Mabuchi 1986). As to the *Protozoa*, in *Tetrahymena* "the division-furrow ring is attached to epiplasm" (Jerka-Dziadosz 1981).

In the cells characterized by a well developed microtubular cytoskeleton, the microtubule organization centres (MTOC) are probably involved in the formation of contractile rings. The anastral character of mitosis and uncertain identification of MTOC in *Amoeba proteus* (Gromov 1985) as well as the disputable or poor development of somatic microtubules in this cell (Christiani et al. 1986) make difficult any speculations about an MTOC-dependent control of its cytokinesis.

The signal initiating the formation of the division furrow, or the "cleavage stimulus" (Rappaport 1968), is transmitted to the cell cortex after anaphase. The nature of the signal is unknown. A role in signal transmission was attributed to the myosin or some of the microtubular proteins, to $Ca^{++}$ ions, and to the polyamines; the concentration of polyamines changes during the cell cycle and reaches maximum just before the division (Kusunoki and Yasumasu 1978), and they activate as well the DNA synthesis as actin polymerization (Oriol-Audit 1978, 1979, Pegg 1986).

One of the polyamines, the spermin, has been used by Gawlitta et al. (1981) as inducer of cytokinesis in *Amoeba proteus*. The intracellular application of this substance, independently of the place of injection, results in formation of a contractile ring, which always cuts off a part of the posterior cell regions, the uroid either alone or together with the adjacent fragment of the trunk. The authors consider this effect as "induced cytokinesis". In the plane of the provoked fission they found the aggregation of thin and thick filaments. Also the IAF-labelled actin accumulated after microinjection within the spermin-induced furrow. The injection of the spermin failed to influence the whole cell cortex as the injected phalloidin does (Stockem et al. 1978), but exerted only a local effect. The work of Gawlitta et al. (1981) does not fill the gap in our knowledge of the distribution of contractile proteins during the normal, post-mitotic cytokinesis in *A. proteus*; nevertheless, it

brings indirect arguments in favour of the accumulation of actin and myosin filaments in the contractile ring of normal dividing amoebae, and the possible role of polyamines as cleavage stimulus in these cells.

Different relationships are known in various types of eukaryotic cells between the position of the cell division plane and the polarity of mother and daughter cells. In ciliates the division is perpendicular to the body axis, that is it separates the rear body end of the anterior specimen from the front of the posterior one. In flagellates the division has no influence at all on the cell polarity, because they divide along the longitudinal body axis. Many polarized animal tissue cells lose their polarity before dividing, but the two daughter cells manifest a mirror symmetry relative to one another, when they separate after fission (Albrecht-Buehler 1977, 1985). *Amoeba proteus* rounds up during mitosis and it is impossible to relate the position of the fission furrow in the dividing sphere in reference to the polarity of the mother amoeba. On the contrary, that can be easily done in reference to the polarity of the daughter amoebae, because the division furrow gives always origin to the uroids of the two arising cells. It seems reasonable to conclude therefore that the components of the contractile ring become consecutively constituents of the young uroids. Especially, the well pronounced accumulation of the thick filaments of highly polymerized myosin just in the contractile ring of dividing amoebae (as inferred from Gawlitta et al. 1981) and in the uroids of locomoting ones (demonstrated by Stockem et al. 1982) may be considered as resulting of such filiation of intracellular structures and functions. This and some other aspects of relations beween the cell division and cell locomotion in amoebae were discussed in the first part of this review (Grębecka 1988). In general, it should be concluded that the existence of a clear morphological and functional polarity of amoebae from the earliest moments of their autonomous life, is certainly a product of the organization assumed by the motor system during the cytokinesis.

## Capping

The formation of caps (capping) is a manifestation of lateral mobility of the surface receptors, which may aggregate in patches and then move backwards and accumulate on the posterior pole of the cell. Usually capping is provoked by cross-linking the receptors by multivalent ligands. Most commonly the lectins and immunoglobulins are used to induce it, however, the backward transport of other molecules (as some dyes) or particles bound to the cell surface presents too all the features of capping.

The first description of the capping and its name are due to Ray (1951), although its re-discovery twenty years later by Taylor et al. (1971) is better known. Since that time the phenomenon of capping was extensively studied and the attempts to reveal its mechanism were undertaken with various techniques in many laboratories (see the reviews by Oliver and Berlin 1982, Yahara 1982, Bourguignon and Bourguignon 1984). Capping is manifested only by the motile cells and, consequently, it is investigated mostly in lymphocytes, macrophages and fibroblasts. It is astonishing how little attention was paid after Ray's paper on *Hartmanella* to the manifestations of capping in *Protista*. Preston and King (1984) observed cap-like aggregation of flagellate bacteria by the cell surface of *Acanthamoeba castellanii*. Two descriptions of capping produced in amoebae by classical inducers were given, by King and Preston (1977): capping of an immunoglobulin by the amoeboid stage of *Naegleria gruberi* and Taylor et al. (1980 a,b): capping of concanavalin A by *Chaos carolinensis*. In 1986 Kukulies et al. analyzed the adsorption and internalization of fluorochromed cationic ligands (including fluorescent lectins) in *Amoeba proteus*. All these ligands were internalized by pinocytosis, concentrated in the uroidal region and finally sequestrated into the surrounding medium by cell constriction. The opinion of Kukulies and his co-workers was that "the ConA receptors in *Amoeba proteus* participate to some extent in induced pinocytosis" though the described phenomenon looked rather as a kind of "internal capping". Nevertheless, the transport of two other kinds of extracellular material by the surface of this amoeba fulfils the requirements of the definition of capping. The vital dyes specifically binding to the mucopolysaccharides of glycocalyx (Neutral Red, Alcian Blue and Ruthenium Red) are gradually accumulated on the surface of the uroid, as reported by Goldacre and Lorch (1950), Prescott (1953), Chapman-Andresen (1964), Czarska and Grębecki (1966), Grębecki (1986). Many particulate surface markers (carmin and carbon particles, glass powder, glass hairs, calcium oxalate and hydroxylapatite crystals, latex beads) may display complicated patterns of surface movements but eventually they form clumps on the tails of locomoting amoebae, as described by Goldacre (1961), Chapman-Andresen (1964), Czarska and Grębecki (1966), Stockem (1966), Wohlfarth-Bottermann and Stockem (1966), Grębecki (1984, 1985, 1986, 1987).

Concerning the mechanism of capping most widely is accepted the theory of backward hauling the ligand-receptor complexes by the cytoskeletal cortical actin filaments bound to the membrane proteins (de Petris and Raff 1973, de Petris 1977). Some authors postulate

that the receptors are transported either by a bulk backward flow of membrane lipids (Bretscher 1976, 1984), or propelled by surface waves (the surf-riding theories of Hewitt 1979 and Berlin and Oliver 1982). In the study of tissue cells many arguments have been accumulated in favour of the first explanation, the cytoskeleton-dependent mechanism of capping:

(1) Blocking actin polymerization by the cytochalazin B inhibits the cap formation in most cells; exceptionally, however, this drug may enhance capping in some tumor or normal cells (see the discussion in Bourguignon and Bourguignon 1984).

(2) Accumulations of actin filaments were found by many authors (e.g., Bourguignon and Singer 1977, Taylor et al. 1980 b, Wang et al. 1982) attached to the cytoplasmic side of the cell membrane under the caps. Actin polymerization is increased during capping (Laub et al. 1981). Subcap aggregations of myosin (Schreiner et al. 1977, Braun et al. 1978 a, Bourguignon 1980) and actomyosin (Condeelis 1979, Paulin and Forest 1981) were also described.

(3) Extraction of the cytoskeleton of capped cells with non-ionic detergents puts in evidence the association of the receptors undergoing capping with the cytoskeletal actin; that was demonstrated, for example in *Dictyostelium* amoebae (Condeelis 1979) and lymphocytes (Bourguignon and Bourguignon 1981) in the formed caps, and even at the patching stage (Bourguignon and Singer 1977). Ash et al. (1977) suggested that even the clustering promotes interaction of these membrane proteins with the cytoskeleton. It accords very well with the agglomeration in the subcap region of $\alpha$ actinin (Geiger and Singer 1979) and fodrin or ankyrin (Bourguignon and Bourguignon 1984), which may be involved in linking actin to membrane proteins.

(4) Schreiner and Unanue (1976 a, b) and Braun et al. (1978 b) have suggested that local anaesthetics can inhibit capping and pinocytosis by displacing membrane $Ca^{++}$ and affect the membrane-cytoskeleton links (according to Nicolson et al. 1977, see the discussion of this point in the first part of this review, Grębecka 1988). Capping is inhibited also by another polar solvent, DMSO, which is known as well as uncoupler of actin-membrane association (Filosa and Fukui 1981, Filosa and Cusato 1986). According to Karnowsky the reversible association between actin and transmembrane protein(s) is Ca-sensitive; cross-linking of surface receptors by ligands and patching could, therefore, simultaneously associate them to the actin microfilaments and liberate calcium needed for the contraction, providing for their transport toward the cap (Klausner et al. 1980).

(5) A specific, but very spectacular and unequivocal evidence of the involvement of actin in the capping of surface receptors, was provided by the study of Heath (1983) on chicken fibroblasts, in which the arcs of microfilaments travel centripetally toward the nucleus. In a monovalent antiserum ligand there is no cross-linking, no movement and no segregation of the surface receptors over the cytoskeletal arcs. When the cross-linking is produced by a supplementary inducer, the receptors aggregate in patches over the arcs, travel centripetally with them and form the cap in the perinuclear region.

The role of actin microfilaments in capping is certainly very well documented. The possibility of microtubules and/or intermediate filaments involvement in this phenomenon remains much more hypothetical, though it was discussed (Albertini and Clark 1975, Yahara 1982, Bourguignon and Bourguignon 1984) and the presence of tubulin (Gabbani et al. 1977) and vimentin (Traub 1985) was reported in the subcap region. As far as *Protista* are concerned, it should be mentioned that, not exactly the capping but at least a directional transport of particulate markers, along the surface of filopodia of foraminiferans, is dependent on microtubules on the opposite side of the plasma membrane (Bowser et al. 1984, 1985).

In *Amoeba proteus* the formation of caps may depend only on the contractile cortex built of actin microfilaments. As it was said above the capping of lectin and immunoglobulin specific receptors by this species of amoeba remains to be examined. However, the formation of caps by extracellular particles adhering to the surface of *A. proteus* is well studied and explained exactly on the same basis as the receptor capping in other cells. The ectoplasmic cytoskeletal layer in locomoting amoeba is steadily retracted toward the actual substratum-adhesion sites (Grębecki 1984, 1985), and the particles adhering to the surface move in the same direction in unison with the ectoplasm, because they are probably attached to the microfilamentous cortex and hauled by it (Grębecki 1986, 1987). Finally, they accumulate on the tail, because the ectoplasm of amoeba moves slower than the cell as a whole.

The dependence of capping on the cell nucleus is another feature relating this phenomenon to the mechanisms and problems of the amoeboid locomotion. As it was stated in the first part of this review (Grębecka 1988), *Amoeba proteus* cannot locomote without the nucleus, which is needed for maintaining the motor polarity of the cell. In the motile tissue cells, enucleated by the cytochalazin B (Shay et al. 1974, 1975, Goldman et al. 1973, 1975), by cutting (Goldstein et al. 1960) and by heat shock (Malawista and De Boisfleury Che-

vance 1982), the cytoplast devoid of the nucleus was, in contrast to amoebae, always capable to locomote, whereas the karyoplast was in most cases immobile or less motile. The capability of both fragments to form caps was studied by Berke and Fishelson (1976), and Ottes-kog et al. (1981) in fibroblasts and leukocytes pretreated with the cytochalazin B. It has been concluded that capping is impossible in the cytoplast, while the karyoplast (nucleus plus residual thin rim of cytoplasm and plasma membrane) is able to cap normally. Apparently, in these cells the nucleus is not needed for locomotion but required for capping. However, these conclusions are subject to caution, because they may be due to artifact provoked by the application of the cytochalazin B, which interferes with actin polymerization and consequently, disturbs as well the locomotion as capping. It should be promising to re-examine the nucleus-locomotion-capping interrelations in *Amoeba proteus*, which may be easily enucleated without the artifactual administration of drugs.

## Pinocytosis

The pinocytosis and capping look, at the first sight, very similar to one another in many features. Both phenomena are based on the interaction of submembraneous contractile proteins with the cell surface. As well the predominant theory of the mechanism of capping (discussed in the precedent chapter) as the widely accepted models of pinocytosis in the proteus-type amoebae (Klein and Stockem 1979, Taylor et al. 1980 a) invoke membrane-cytoskeleton interactions. Moreover, in both cases the distribution of the induced events and structures is not determined by the localization of the acting stimulus, but by the innate polarity of the cell.

On the other hand, however, a basic difference between the capping and pinocytosis is related to the time at which the role of cell polarity becomes manifested. In the case of the capping, the molecules of the ligand evenly dispersed in the medium are randomly bound to the cell surface (providing the receptors are distributed at random), then the ligand-receptor complexes still randomly form patches and clusters, and only in the final issue the arising cap occupies the polar position. In pinocytosis, the order of manifesting the cell polarity is inverted. The first pinocytotic pseudopodia appear at the posterior body pole, next on the surface of retracting fronts, and after several minutes they uniformly cover the whole cell surface (Grębecka and Kłopocka 1985). At the threshold concentration of the inducing agent the pinocytotic response is produced only by the uroid of amoeba (Chapman-Andresen

1962). We state in general that the cell polarity is manifested at the final stages of capping and, on the contrary, at the initial steps of pinocytosis.

It is not easy to understand the background of this difference between two phenomena which may often be functionally coupled (the endosomes are as a rule produced beneath the cap). De Petris (1977) concluded that: "In contrast to capping, a phenomenon, which involves the entire membrane, pinocytosis is local phenomenon with presumably "segmental" characteristics similar to those observed in phagocytosis, which involves a limited region of the membrane". However, the validity of this statement for amoebae is disputable. It may apply to a single pinocytotic pseudopodium with its channel, but it ignores the pinocytotic response of the cell as a whole, which in the case of *A. proteus*, may involve the entire surface with the preferential manifestation at the posterior body pole. It should be added that in some other amoebae (e. g., in *Pelomyxa palustris*, according to Chapman-Andersen 1971, 1977) the endocytosis takes place only at the uroid of the cell.

The major deal of the phenomenological research on pinocytosis of the large fresh water amoebae has been done in the fifties and sixties by Holter and Chapman-Andresen. Recently this trend of research with more pharmacological approach, is continued by Josefsson and his coworkers. The study of the ultrastructural background of pinocytosis of free living amoebae was, in the past two decades, concentrated mainly in the group of Wohlfarth-Bottermann and Stockem.

There are two forms of pinocytosis known in *Amoeba proteus*. One of them, similar to that described in the tissue cells, is limited to the formation of pinocytotic channels and endosomes, another one involves the formation of specific pinocytotic pseudopodia, each of them developed around one invaginating channel. According to the mode of manifestation of these two phenomena in amoebae they are respectively called the permanent, and the induced pinocytosis (Fig. 1 a, b).

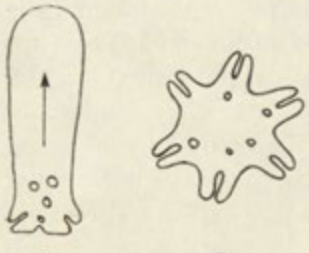


Fig. 1. Permanent (A) and induced (B) pinocytosis in *Amoeba proteus* (redrawn after Stockem 1969)

The Permanent Pinocytosis

Roth (1960) was the first to describe the presence of pinocytotic vesicles in the amoebae which were not prealably exposed to any inducer. The presence of such endosomes was evidently due to a spontaneous pinocytotic process. The same phenomenon has been called permanent pinocytosis by Wohlfarth-Bottermann and Stockem (1966), because in contrast to the induced pinocytosis, it may be continuously produced without interrupting the cell locomotion. The spontaneously arising channels are present only in the uroids of migrating amoebae and vary in dimensions, from those seen in the light microscope (Fig. 1 A) to the smallest ones detectable by electron microscopy. The permanent pinocytosis is certainly the most important component of membrane recycling in amoebae, although it produces smaller and less numerous invaginations than the induced pinocytosis. The uptake of the surface membrane by the permanent pinocytosis was estimated to represent 6-12% per 1 h in *Amoeba proteus* (Wolpert and O'Neill 1962) and 1% in *Chaos carolinensis* (Bruce and Marshall 1965), while during the induced pinocytosis *Amoeba proteus* may internalize 50% of its surface material in 20 min. (Chapman-Andresen 1962). It may be suggested that in the course of permanent pinocytosis a moving amoeba internalizes in the rear as much membrane as it emerges to the surface again in the frontal zone but during the induced pinocytosis the membrane intake exceeds membrane renewal (Chapman-Andresen 1977).

It may be concluded as well, that the contractile apparatus is involved in the permanent pinocytosis only locally and in a lesser extent than in the induced pinocytosis, since in the first case the cell migration is not affected and in the latter is completely interrupted.

The only attempt of explaining the polar localization of the permanent pinocytosis in the uroid of *Amoeba proteus* was made by Josefsson (1968). According to his concept it is induced, as usually, by monovalent cations. Their concentration in the glycocalyx is 21 times higher than in the medium (Hendil 1971). Josefsson presumes that this concentration may be inefficient in the presence of relatively high level of Ca at the cell surface, but it may provoke pinocytosis if the Ca concentration decreases. According to Shida (1970) the concentration of calcium on the uroid is lower than on the other surface areas of amoeba. So, this explanation is based on the idea that the permanent pinocytosis is also externally induced, depends on the antagonism between the inducers and calcium and, eventually, on a lesser amount of Ca bound to the uroidal surface.

However, the Ca defficiency on the uroid described by Shida (1970), and quoted after him by Josefsson, has not been confirmed by other authors. The aequorin luminescence on the surface of *Chaos carolinensis* (Taylor et al. 1980 a) is most bright and steady at the tail region. In *Amoeba proteus* exposed to the inducers of pinocytosis the CTC fluorescence, originally dispersed along the surface, becomes concentrated within 30 s exclusively in the pinocytotic channels (Gawlitta et al. 1980). According to Stockem and Klein (1979) in the control freely locomoting amoebae Ca is attached to the cytoplasmic side of the plasma membrane and detected mainly in the uroid region. So, the assymetry of Ca distribution, as well between the two sides of the plasma membrane as between the two cell poles of a moving amoeba, are opposite to those described by Shida (1970) and involved in the explanation of the permanent pinocytosis proposed by Josefsson (1968).

Certainly, the manifestation of the spontaneous permanent pinocytosis at the posterior body pole of migrating amoebae is related to some, not yet defined, specific features of the "uroidal milieu". It might be therefore suggested to look for the manifestation of spontaneous pinocytosis also in the retracting pseudopodia, because they reproduce the same structural and functional conditions which characterize the uroids (and because they are almost as sensitive as the uroids to the pinocytotic inducers — Grębecka and Kłopocka 1985). Among the uroidal features which speculatively may be suspected to have a relation to the permanent pinocytosis, one could enumerate for example: the regular presence of highly polymerized myosin filaments, the sporadic occurrence of actin microfilament bundles (Taylor et al. 1980 b), the low actin mobility (Wang et al. 1982), the differences in the free and membrane-bound Ca concentrations which were mentioned above, the difference in the membrane potential between the posterior and anterior body pole of moving amoeba (Nuccitelli et al. 1977). The importance of the last factor is stressed by the findings (Josefsson et al. 1975) that during the pinocytosis of amoeba its membrane resistance is decreased and the membrane potential falls near zero. Finally, the rich pool of the membrane available for invagination on the strongly corrugated surface of the uroid, which has been discussed by us (Grębecka and Kłopocka 1985) as a factor of the polarity of induced pinocytosis, may be also involved in the manifestation of the permament pinocytosis in the tail region.

Another approach to the interpretation of the spontaneous permanent pinocytosis in amoebae may be attempted in connection with the discovery of the spontaneous capping (Yahara and Kakimoto-Sameshima 1977, Braun et al. 1978 a and b. Bourguignon et al.

1981). For example, Braun et al. (1978 a, b) observed in the migrating lymphocytes the retrograde transport and aggregation on the posterior cell pole of non-complexed Ig and Fc receptors, in the absence of any ligand; addition of the respective antibodies induced patching and accelerated capping. On the other hand, it is commonly known that endocytotic vacuoles are always present beneath a cap. It may be suggested therefore that the spontaneous permanent pinocytosis at the rear end of a locomoting cell, such as is described in *Amoeba proteus*, may be initiated by the mechanism of capping and be a factor of the continuous, spontaneous recycling of the surface receptors.

The Induced Pinocytosis and Phagocytosis

The induced pinocytosis, in contrast to the permanent one, is characterized besides the invagination of channels, also by the formation of pinocytotic pseudopodia (Fig. 1 B). This type of pinocytosis is a much more complicated process thought to consist of several interrelated events (some of them are of course common to both phenomena).

The first step consists in binding the inducing molecules by the cell surface of amoeba (Stockem and Klein 1979). The inducers different in structure may react in different way with the surface (Hendil 1971), which involves the existence of various pinocytosis inducing sites on the amoeba surface (Prusch 1986). In general, the solutes provoking pinocytosis have a net positive charge and, therefore, they probably interact only with negative surface sites. In *A. proteus* the association of such cationic inducers to the respective binding sites (Brandt 1958, Schumaker (1958) triggers a class of following events: increasing of membrane conductance, increasing of membrane permeability, decreasing of membrane potential (Brandt and Freeman 1967, Josefsson 1966, 1968, Josefsson et al. 1975, Braatz-Schade 1978), followed by displacement of a part of the surface associated $Ca^{++}$ (Josefsson 1975, Prusch and Hannafin 1979).

Owing to these initial changes, the concentration of free Ca in the submembraneous space transiently increases (Allison 1973, Josefsson 1975). It may be produced as well by displacing the bound Ca from the inner side of the plasma membrane as by penetration of the extracellular Ca across the membrane (Josefsson 1975, Stockem and Klein 1979, Gawlitta et al. 1980, Prusch 1986). The local increase of $Ca^{++}$ concentration initiates the contractile activity of the filamentous cortical layer resulting in the formation of a pinocytotic channel (Allison 1973, Allison and Davies 1974, Cohen and de Vries 1973, Josefsson 1975, Klein and Stockem 1979, Taylor et al. 1980 a, Stockem et al. 1983 a, Stockem et al. 1983

b, Grębecka and Kłopocka 1987). An excellent scheme of the early stages of induction of pinocytosis in amoebae was given by Taylor et al. (1980 a). It clearly shows the local aggregation and orientation of microfilaments, their link with the plasma membrane and anchoring to the cortical gel layer (Fig. 2 A). Next, as Taylor says, "the membrane remains attached to the contractile fibril at the base of the channel and is pulled down into cortex during the contraction". In that way the pinocytotic channel is presumably invaginated.


Fig. 2. Arisal of a pinocytotic pseudopodium, after Taylor et al. 1980 a (A) and a locomotory pseudopodium (B), in *A. proteus*. Note in both the disengagement of the contractile layer from the cell membrane, which results in the protrusion of either type of pseudopodium

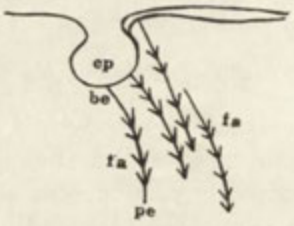


Fig. 3. The coated pits redrawn after Salisbury 1980. Note the presence of actin filaments (fa) attached to the coated pit (cp) by their barbed ends (be). The pointed ends are directed toward the cell interior

Generally similar are the informations concerning the receptor mediated pinocytosis in the tissue cells. In the coated pits which are the sites of intense pinocytosis, the actin filaments are aggregated and attached to the plasma membrane.

Moreover, they are ordered and centripetally orientated (Fig. 3), which is supposed to present the functional state of the filament system involved in the invagination of channels and inward transport of endosomes, via a sliding filament interaction between actin and myosin in the cortex (Salisbury et al. 1980, Condeelis 1981).

Also, in amoebae the aggregation of submembraneous actin filaments is accompanied by the parallel condensation of the surface coat, as well in the pinocytizing specimens (Taylor et al. 1976) as in the phagocytizing ones (Jeon and Jeon 1983), which is probably strictly analogous to the coated pits of the tissue cells. It seems that in *P. palustris* the mucous layer is more or exclusively developed at the uroid. This is interesting in view of the observation that in this species normal and induced endocytosis occurs only in the uroid (Chapman-Andresen 1973). The coated pits and in general the local condensation of the surface coat, as well in amoebae as in the tissue cells, may indicate that patching of activated receptors is a necessary early step of the induced

pinocytosis and phagocytosis, like in capping. The probable identity of the first events during the capping and during the induced forms of endocytosis remains to be explored.

The immunofluorescence study of actin distribution in pinocytizing *Amoeba proteus* (Taylor et al. 1980 b, Stockem et al. 1983 a, b) revealed its abundance just below the channel. This finding correlates well with the distribution of Ca in the pinocytizing amoebae, put in evidence by the CTC fluorescence (Gawlitta et al. 1980): at the stage of separation of the endosomes the fluorescence is mainly concentrated at the basal region of the channel. According to Juliano et al. (1971) and Nicolson et al. (1977) the Ca is required to produce stable filament-membrane attachments. If so, the parallel accumulation of both, actin and calcium, at the bottom of pinocytotic channels may indicate the firm anchoring of the plasma membrane to the contractile system and perseverance of the pulling activity.

The phenomena described above are more or less similar in different cells and in different types of pinocytosis. Apparently they fulfil all the requirements needed to enable the internalization of a portion of the fluid medium or a number of the receptor-bound molecules. Nevertheless, the large free living amoebae develop during the pinocytosis one peculiar structure more: the pinocytotic pseudopodium. Its arisal is also outlined in the scheme proposed by Taylor et al. (1980 a) (Fig. 2 A). In the vicinity of the aggregated and membrane-attached filaments, around the proper invagination site, a clear zone is seen, in which the regular actin layer lost the contact with the plasma membrane. Such disengagement of the motor system from the cell membrane results in the protrusion of the pinocytotic pseudopodium. According to Taylor: "the contracting fibrils separate from the plasmalemma and force the solated cortex out...". It should be reminded that it is almost the same mechanism by which (Fig. 2 B) the locomotory pseudopodia are initiated and developed (for the references see the first part of this review — Grębecka 1988). Moreover, in the active pinocytotic pseudopodia the contractile cortical layer is periodically withdrawn and rebuilt beneath the membrane, like in the fronts of locomotion. These phenomena in the locomoting and pinocytizing amoebae were demonstrated by immunofluorescence techniques (Stockem et al. 1983 a, b) and by cinematography of cells pretreated with a heat shock (Grębecki and Kwiatkowska 1988). It appears that not only the involvement of the motile apparatus, but also the manifestation of some essentially motor functions in the course of the induced pinocytosis, may serve to relate this phenomenon to some aspects of locomotion.

The cooperation of the motor functions of the pseudopodia with the

interception and internalization of the extracellular material is further developed in the case of phagocytosis. The parallel between the induced pinocytosis and phagocytosis has been already outlined by Brandt and Pappas (1960). Unequivocal conclusions were drawn in this respect by Bowers (1977), from the study of these two types of endocytosis in *Acanthamoeba castellanii*: "in *Acanthamoeba* phagocytosis and pinocytosis are effected by the same molecular mechanisms within the cytoplasm and have a common control mechanism".

Recently, comparative studies of phagocytosis and pinocytosis, with special reference to the role of Ca in the two phenomena, were carried out in *Amoeba proteus* (Prusch and Minck 1985, Prusch 1986). The authors point out that in both cases the intake of the extracellular material is associated to morphologically similar behaviour of the cell membrane, with involvement of the surface receptors, and is regulated in the same way by $Ca^{++}$. The increase of the free Ca concentration up to $10^{-4}$ M stimulates as well phagocytotic as the pinocytotic activity of amoebae, but its further increase beyond this critical concentration level brings inhibitory effects (it is supposed that in high $Ca^{++}$ concentrations this ion substitutes for the inducer molecules at the surface receptor sites). Prusch is, however, much less cathegorical than Bowers in presenting common features of phagocytosis and pinocytosis, because in his opinion both reactions are mediated by the different surface receptors (in fact, the gelatin induced only pinocytosis and glutathione only phagocytosis in the experiments of Prusch an Minck 1985). But it may be prematured to generalize about the existence of two different sets of receptors specialized in one or another form of endocytosis. Receptors recognize other properties of inducers, rather than their soluted or particulated state.

The striking morphological parallel between these types of endocytosis in amoeba is well demonstrated by the classical picture from


Fig. 4. Morphological homology between the induced pinocytosis and phagocytosis in *A. proteus* (from Chapman-Andresen and Prescott 1956, combined with other sources). A — the pinocytotic channel produced in virus suspension, B — the cavity produced in methionine solution, C — the food cup. The broken line shows the contractile layer beneath the invaginated membranes (according to Taylor et al. 1980 b, Stockem et al. 1983 a, b and Jeon and Jeon 1983)

Chapman-Andresen and Prescott (1956) (Fig. 4), of the pinocytotic channel produced in the methionine solution, the "bottle-shaped cavity" induced by the presence of the tobacco mosaic virus, and the

food cup containing a small ciliate. The engagement of the motor system of amoeba either in pinocytosis or in phagocytosis, leads in both cases to the cessation of locomotion. The actin distribution during phagocytosis is essentially similar to that described earlier for the pinocytizing cells. The thick and condensed layer of microfilaments beneath the membrane of the food cup (like around the pinocytotic channels) has been demonstrated by electron microscopy (Christiansen and Marshall 1965 and Jeon and Jeon 1983). The action in the phagocytizing *Amoeba proteus* was also identified by immunofluorescence which demonstrated "local polymerization of actin at the tip of pseudopodia forming the food cup and around the nascent phagosome" (Stockem et al. 1983 b). The actin layer beneath the membrane of the food cup was also found by Yumura et al. (1984) in *Dictyostelium* amoebae.

The phagocytosis in the tissue cells is also strikingly similar to the pinocytosis manifested by them. For example, the description of the initiation of phagocytosis in macrophages given by Silverstein et al. (1980) (Fig. 5) could equally well apply to either one of the two cases of endocytosis: "The initial ligand-receptor interaction generates a transmembrane signal that initiates the assembly of contractile proteins."

Fig. 5. The accumulation and aggregation of contractile filaments during the phagocytotic induction (after Silverstein 1980)

Certainly, the elementar common feature of the induced pinocytosis and phagocytosis is the need for an external stimulating agent to initiate them, in contrast to some other manifestiations of cell motility which may be spontaneous. But perhaps one of the principal differences between the pinocytosis and phagocytosis is also based on the mode of their induction. The induction of phagocytotic pseudopodia is limited to the contact area between amoeba and the prey. Phagocytosis is accompanied by an increase in area of cell surface contacting the substratum, whereas during pinocytosis a sharp decrease of contact is observed (Opas 1981). The prey polarizes the cell in a new direction (probably by chemotaxis — Taylor et al. 1982, Prusch and Minck 1985), at least in the initial stage of phagocytosis (before the cessation of locomotion in the ingestion phase). The pinocytosis is, on the contrary, induced

under the conditions of uniform contact of the whole cell surface with the stimulating factor. Since, however, the pinocytotic pseudopodia are not developed at random, but follow a certain order along the body of amoeba, we should refer for explanation to the pre-existing polarity of the cell, established in the course of locomotion.

In the locomoting specimens of *Amoeba proteus* subject to such inducers as for example $Na^+$ or heparin, the pinocytotic pseudopodia always arise first in the uroidal zone, then in the former frontal area when it begins to be retracted, and eventually they cover the whole surface of the cell, which in the meantime had lost its earlier locomotory polarity and assumed instead the form of the pinocytotic rosette (Grębecka and Kłopocka 1985, 1986). It was also observed earlier by Chapman-Andresen (1963) that at low concentrations of inducers the whole pinocytotic response may be limited only to the uroidal region. Also in KCN treated nucleated pinocytizing fragments of *Amoeba proteus*, which in general revealed a very poor pinocytotic reaction, "the channel arising mostly in the tail region" (Hrebenda 1986) was seen.

Two known factors, and the third hypothetical, may contribute to this pattern of manifestation of the induced pinocytosis: the characteristic oscillations of the endoplasmic streaming arising after the application of the inducer, the uneven polar distribution of the surface membrane pool disposable for internalization, and the pre-existence of the channels of permanent pinocytosis at the posterior body end, which may be due to a backward transport of surface receptors.

Immediately after the administration of inducer to a locomoting amoeba the direction of the endoplasm streaming is reversed; thereafter, many forth and back oscillations of the internal flow may occur until the amoeba is transformed into a rosette. According to Klein and Stockem (1979) these oscillations are related to the distinct phases (or components) of the development of pinocytotic pseudopodia with channels. It seems that channels invagination and pulling inward is reinforced at the periods of the endoplasm outflow to other cell regions. Conversely, the pinocytotic pseudopodium as a whole is further extended when the endoplasm flows in. The polar manifestation of the induced pinocytosis is therefore in some extent related to the differences between its two components: the purely endocytotic component (the pulling force directed inward) and the accesory locomotor element (the pressure force directed outward). Initially, the endoplasm outflow from the uroid favours the invagination processes, and then, the first streaming reversal after the application of inducer provokes in addition the arisal of pinocytotic pseudopodia at the posterior body pole. At the anterior pole, simultaneously, the endoplasm flows out, the cell surface

shrinks and becomes ready to invaginate. After the next change of the streaming direction, the channel invagination sites produced at the former front of amoeba assume the pseudopodial shape and structure. Eventually, the polar pattern of pinocytosis is gradually replaced by its random manifestation, when the streaming pattern becomes disorganized and the cell rounds up (Fig. 6).


Fig. 6. The sequence of events during the induced pinocytosis in *A. proteus*. A — normally moving amoeba, B — arising of pinocytotic pseudopodia only in the uroid, C — pinocytotic pseudopodia develop at the anterior pole, D — the final rosette stage. Note the changes in membrane folding (according to Grębecka and Kłopocka 1985) and streaming pattern (according to Klein and Stockem 1979)

As it was mentioned in the first part of this review (Grębecka 1988), the frontal part of a migrating amoeba is smooth, whereas in the posterior region the cell surface is strongly corrugated. On the other hand, as it was already pointed out by Chapman-Andresen (1973), the availability of sufficient pool of the membrane to be invaginated and internalized (that is, a high surface to volume ratio) is a necessary precondition of pinocytosis. It was later demonstrated by us (Kłopocka and Grębecka 1986) that the surface membrane pool is not the universal, however, the ultimate factor limiting the induced pinocytosis. The differences in the distribution of the surface membrane pool between the two body poles of normal amoeba are provoked by the continuous membrane unfolding in the advancing cell parts and its refolding in the withdrawing regions (Czarska and Grębecki 1966, Haberey et al. 1969, Stockem et al. 1969). We postulated therefore (Grębecka and Kłopocka 1985) that the induced pinocytosis is first manifested at the posterior body pole of amoeba, bacause in that area the invagination is from the very beginning much easier, owing to the high folding degree of the surface. The former fronts are next to produce pinocytotic channels and pseudopodia, because they are retracted under the influence of the inducer and their surface is then refolded. This concept has been positively tested in the monotactic and polytactic individuals (which differ by the steepness of the surface folding gradient), and by dissecting amoebae into fragments characterized by different de-

gree and distribution of surface folding and surface to volume ratio (Fig. 7).

Finally, it may be suggested as a working hypothesis, that the start of the induced pinocytosis at the uroid depends on the presence of ready channels which were produced there earlier by the permanent pinocy-

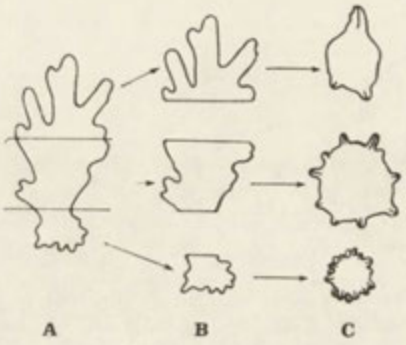


Fig. 7. *Amoeba proteus* (A) dissected into fragments (B) characterized by different surface to volume ratio. Note in (C) the different number of pinocytotic pseudopodia relative to the origin of fragment (after Grębecka and Kłopocka 1985)

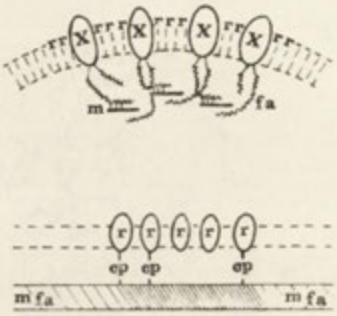


Fig. 8. The mechanism of capping proposed by Bourguignon and Bourguignon (1984) (top), and the mechanism of pinocytosis after Grębecka and Kłopocka (1987) (bottom); r — receptor molecules, x and cp — connecting proteins, m — myosin, fa — F-actin. Note the interconnections and accumulation of cortical proteins and other components

tosis. Taking into account the presumable links between the permanent pinocytosis and capping (see p. 188) we could further expect that at the first stage of induction of pinocytosis, when the locomotory polarity of amoeba is not yet effaced, the activated pinocytotic receptors are transported backwards. It means that the posterior localization of the permanent pinocytosis and of the initial stage of induced pinocytosis may both be dependent on the mechanism of capping (Fig. 8). Hovewer, the possibility that a pinocytotic receptor may be ingested far away from the place of its activation by the inducer molecule, is quite new and open to examination.

## Some General Conclusions

The present state of the knowledge of relations between cell polarity and various cell functions which involve movement or have a motor component, as far as *Amoeba proteus* and/or other amoeboid cells are concerned, may be summarized in the following way:

(1) As well the capping as pinocytosis, either the permanent or induced one, have principally the same molecular background.

(2) The initial stages of all these phenomena are based on the same mechanism: interaction of the ligand molecules with the surface receptors, followed by the aggregation of actin filaments and tightening their links with the plasma membrane at the activated sites.

(3) The morphological manifestation of these reactions and their distribution along the cell surface seem to depend in the large extent, like the motor polarity and locomotion, on the co-existence (and often the interlacing) of the sites characterized by condensation and strong membrane-attachment of the motor system with those at which the contractile apparatus is distended and may loose its association with the plasma membrane.

(4) The localization of capping and permanent pinocytosis are strictly related to the locomotory movements. The migrating amoebae manifest them only at the posterior cell pole. The unattached ones which cannot locomote, display, however, the same posterior localization of capping and permanent pinocytosis, provided the intracellular movements maintain the motor polarity of the cell without effective locomotion.

(5) The induced pinocytosis may occur in non-locomotive and unpolarized cells. Nevertheless, its manifestation has initially a polar character, if a migrating amoeba is exposed to a moderate inducer. The sequential development of pinocytotic pseudopodia at the two cell poles is observed as long as the intracellular streaming pattern and the surface folding degree remain distributed and integrated in the polar manner. The involvement of the motor system in producing the induced pinocytosis is enough extensive to inhibit completely the locomotion at the next stages (in contrast to the permanent pinocytosis and capping).

(6) The molecular background and the mechanism of phagocytosis is almost identical to those of the induced pinocytosis. It basically differs, however, from the latter by cell polarization orientated toward the strictly localized inducing factor. At the first steps of phagocytosis the cell polarity changes may be associated to chemotaxis and surface contact phenomena.

(7) The cytokinesis, as a periodical phenomenon limited in time, reorganizes the motor system and motor polarity of amoeba and dominates any other movements and reactions. But in the final issue it determines and restores the motor polarity of the daughter cells.

(8) The locomotion is in amoebae the only motor phenomenon practically never interrupted during the whole cell life, which continuously creates and maintains the dynamical but always specified patterns and spatial distribution of all structures and functions from the molecular

up to the macromorphological level. As a result all the phenomena manifested by the cell of amoeba must be and are, on one or another way, locomotion-dependent.

(9) It seems possible that in *A. proteus* the mechanisms controlling the contractility and motility are rather more similar to those operating in the metazoan cells than in other Protista.

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# Action of Quinine Sulfate on the Chemosensory Responses of the Ciliate *Paramecium caudatum*, to Inorganic Cations

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*Synopsis.* Quinine, at low concentrations ($\leqslant$ 8 μM) acts as potent inhibitor of $K^+$ induced continuous ciliary reversal (CCR) for *Paramecium* cells tested in solutions containing moderate concentrations (0.016 to 0.031 mM) of $CaCl_2$ at pH 7.1. At the same concentrations, quinine has little effect on either NaCl-or $BaCl_2$-induced periodic ciliary reversal (PCR) behavioral responses. The relative potency of quinine as an antagonist of $K^+$-induced CCR is inversely related to the extracellular calcium levels. Quinine also prolongs (or prevents) the cells from returning to forward swimming after induction of partial ciliary reversal (PaCR) by $K^+$ ions and, at higher concentrations ($\geqslant$ 8 μM), markedly augments the $BaCl_2$-induced PCR response so that the cells swim backwards for prolonged periods of time. Quinine was toxic to cells in bacterized hay-infusion cultures (containing $\sim$ 0.014 mM total calcium) with growth inhibition observed at $\geqslant$ 2 μM. The effects of quinine do not appear to be a phototoxic effect since cell motility and behavior was unaffected by various illumination conditions. The mechanisms of action of quinine are discussed in terms of its being a cationic organic base (to block the induction of ciliary reversal by $K^+$) and as an inhibitor of voltage- or $Ca^{2+}$-activated outward $K^+$ currents (to prolong renormalization following KCl stimulation and augment the $BaCl_2$-induced PCR responses).

The quinine alkaloids have been widely reported as being very toxic to ciliate protozoa such as *Paramecium* (Acton 1922, Brahmachari et al. 1930, Cantacuzene 1925, Crane 1921, Dixon and Premankur 1927, Dryl 1961, Dryl and Kurdybacha 1978, Feiler 1927, Potts 1944, Sarkar 1936) and *Tetrahymena* (Henry and Brown 1923). Quinine (or its isomer, quinidine) have also been used

for many years as "chemorepellents" for *Paramecium* in that the organism, when presented with a choice of a salt solution or tap water versus a solution containing quinine, avoids swimming into the quinine-containing solution (Dryl 1959, 1961, Dryl and Kurdybacha 1978, Van Houten et al. 1975). In addition, quinine has been used as a "bitter stimulus" in comparative studies using frog and slime mould systems alongside *Tetrahymena* (Ataka et al. 1978). Quinine has been reported to be able to induce repeated ciliary reversal episodes in *Paramecium* (Dryl and Kurdybacha 1978, Potts 1944, Van Houten et al. 1975) when the cells come into contact with solutions containing quinine and, as a result, the cells show repeated brief episodes of backward swimming (avoiding reactions). Low concentrations of quinine have been reported to slow the swimming speed of *Paramecium* (Dryl 1961) or to induce a "transient increased activity..... on first contact with the drug" (Dixon and Premankur 1972). In addition, quinine was reported to induce what appear to be gyration, spinning and circling motions (Sarkar 1936) (ie., partial ciliary reversal modes: see Doughty and Dryl 1981 for review of these swimming types). Most of these studies were carried out in water, culture medium (hay infusions) or dilute phosphate buffers. At high concentrations (and in the presence of millimolar concentrations of calcium ions), the isomer of quinine (quinidine) has been reported to reduce the magnitude of the resting membrane potential that can be recorded across the surface membranes of *Paramecium* through the use of intracellular microelectrodes (Van Houten 1979). In addition to this depolarizing action, quinidine elicited all-or-nothing spontaneous spike depolarizing discharges from microelectrode-impaled cells (Van Houten 1979).

All such reports clearly indicate that quinine (or quinidine) is capable of inducing ciliary reversal in *Paramecium* by altering the electrical properties of the cells' surface and ciliary membranes. Furthermore, if *Paramecium* are incubated in high concentrations of KCl (20 mM), then they show a markedly reduced sensitivity to subsequent testing with quinine as a "chemorepellent" (Dryl 1959). For *Paramecium* in the absence of quinine or other drugs, stimulation with KCl elicits a characteristic continuous ciliary reversal (CCR) response (Mast and Nadler 1926, Kamada and Kinosita 1940, Oliphant 1938, Grębecki 1964, Kuźnicki 1966, Naitoh 1968, Doughty 1986, Doughty and Dodd 1978). In this response, the cells swim backwards for several seconds and then execute a characteristic series of spinning, circling and looping swimming patterns until forward swimming is regained. Thus quinine itself appears to be able to induce these partial ciliary reversal behaviors and the action of quinine can be attenuated by KCl treatment.

However, a review of this literature does not indicate that the actions of quinine on the direct and immediate responses of *Paramecium* to KCl (or other cations) have been studied.

## Materials and Methods

*Paramecium caudatum* was cultured on bacterized hay infusions as previously detailed (Doughty 1986) with the cultures being maintained under a light : dark cycle of 14 : 10 h starting at 06.00 h. The illumination was provided by normal laboratory lighting (cool white fluorescent tubes) with an incident fluence rate of $\sim$ 0.5 $W \cdot m^{-2}$. Late logarithmic growth stage cells were harvested and washed into adaptation buffer composed of 5 mM MOPS/NaOH, pH 7.1 which contained 0.016, 0.031 or 0.062 mM $CaCl_2$. Cells, at a density of $\sim$ 2500/ml, were then adapted for 18-24 h at room temperature and under the fluorescent lighting prior to use. The culture and all experiments were carried out at 23-25°C.

The toxicity of quinine towards the cells was assessed in three ways. Firstly, quinine sulfate was added to 50 ml of the bacterized culture media and then approximately 10,000 cells added and the cell division followed by taking cell counts every 20-24 h. Cell counting was performed, in duplicate, on 1 ml aliquots to which was added 0.1 ml of 10% w/v formalin and the sample of immobilized cells transferred to a counting chamber. Secondly, small aliquots of adapted cells were added to 1 ml of adaptation buffer in small test tubes which contained various concentrations of quinine sulfate. The cells (final density of $\sim$ 100/ml) were then left either under the laboratory lighting for 24 h or the tubes were placed in a light-proof cupboard. The numbers of motile cells were counted after 24 h and the concentration required to immobilize (kill) 50% of the cells ($LD_{50}$) determined by graphical analyses. These experiments were repeated three times using different cultures. Finally, after conducting tests of the actions of quinine on the responses of *Paramecium* to cations, the cells were inspected at 1,2 and 12 h afterwards.

Cell swimmming motion was qualitatively assessed by transferring small aliquots of adapted cells to 1 ml samples of adaptation buffers (with or without added test chemicals) in 3.5 cm diameter plastic petri dishes (Falcon product No. 3001; Beckton-Dickinson, Mississauga, Ontario). The cells were then observed under a stereo dissecting microscope either under dark-field illumination or under diffuse illumination (ground glass faced mirror). In some cases, cell motion was quantitatively assessed by use of an event marker (equipped with hand and foot-operated controls) with its paper trace running at 5 mm/s. Periods of reversed swimming, transient reversal events (avoiding reactions) and the time intervals between them were thus assessed to 0.1 s accuracy. In most experiments, the swimming paths of 9-15 cells were chosen at random and recorded for 35 to 45 s. The final cell density in all such experiments was around 100/ml. All of these experiments were repeated twice using different batches of culture.

The responses of *Paramecium* to stimulation with inorganic salts were assessed essentially as above by transfer of small aliquots to 1 ml samples of adaptation buffers that additionally contained either KCl, $BaCl_2$ or NaCl with or without various concentrations of quinine. In some cases, small volumes of concentrated stocks of quinine were added to samples of the adapted cells (at a density of

~ 2500/ml) and then aliquots of these pre-treated cells removed for testing. For KCL stimulation, the duration of ciliary reversal behaviors was assessed with the use of a stop watch to 0.5 s accuracy. The responses to $BaCl_2$ and NaCl were assessed with the use of the event marker.

The quinine was freshly prepared daily by dissolving quinine sulfate into the adaptation buffer. These stock solutions, which were kept shielded from light at all times, were then diluted to give the desired final concentrations. The concentrations of salts were achived by the addition of 10 µl aliquots of concentrated stock salt solutions into 1 ml aliquots of the adaptation buffers.

## Experiments

### Effects of Quinine on Growth of *Paramecium caudatum*

Bacterized hay-cerophyll infusions were used in this study. The infusions were prepared with the addition of 5 mM $NaH_2PO_4$ and no calcium salts were added. Analysis of samples of different infusions (by atomic absorption spectroscopy) revealed total calcium of 0.01 to 0.014 mM when the standard routine of 15 min of boiling of the hay-cerophyll mixture was adopted prior to filtration and autoclaving. If the mixtures were boiled for 30 min calcium levels of 0.05 mM were obtained. In common with other cerophyll cultures (Browning and Nelson 1976), the cultures contain phosphate salts so the ionized (free) calcium can be expected to be less than these values since some of the calcium will be complexed by the phosphate (Wetzell 1983).

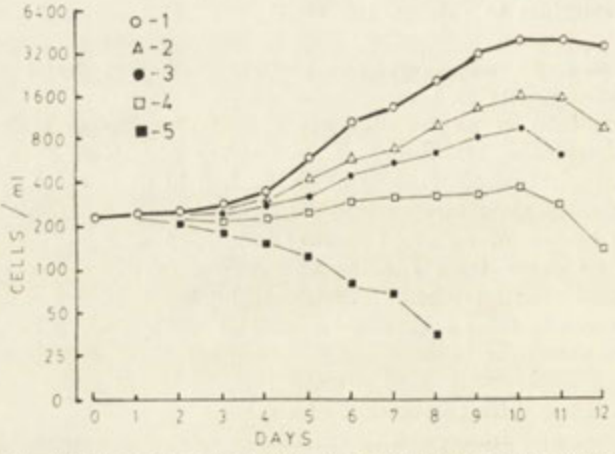


Fig. 1. Growth of *Paramecium caudatum* in bacterized hay infusions in the presence of various levels of quinine. Medium contains ⩽ 0.014 mM calcium. 1 — no quinine, 2 — 2 µM quinine, 3 — 4 µM, 4 — 8 µM and 5 — 16 µM quinine. Results are mean cell counts from two separate cultures at each quinine concentration

Using a standard inoculum of ~ 10 000 cells into 50 ml cultures, the cultures double approximately every 28 h until late logarithmic phase of growth is achieved in 8 to 9 days (Fig. 1). Even trace levels of quinine sulfate (i.e., $\leqslant$ 1 μM) effect a slowing of the cell growth rate and the alkaloid is clearly toxic at concentrations of above 8 μM (Fig. 1). Microscopic analyses of cells from inhibited cultures (1 or 2 μM) indicated that the cells were shorter than normal and had very pronounced contractile vacuoles and a granular cytoplasm. The cell motility in such cultures appeared to be slower and, perphaps as a consequence of this, cells in 8 day-old cultures treated with 2 μM quinine sulfate showed less frequent spontaneous avoiding reactions (AR's). The frequency of avoiding reactions (FAR) was reduced from 11 ± 2/min to 6 ± 2/min (n = 25) while the mean interval between avoiding reactions was increased from 6.8 ± 1.2 s to 9.3 ± 1.2 s

## Effect of Quinine on the Motility of *Paramecium* in Adaptation Buffers Containing Different Levels of $CaCl_2$

The basic adaptation buffer containing 0.016 mM added $CaCl_2$ was chosen because of the results from atomic absorption analysis of the total calcium in the cultures. In such adaptation buffers, the cells displayed the normal forward left spiral motion (FLS) with only occasional spontaneous avoiding reactions (FAR $\leqslant$ 5/min). The cells responded to the presence of quinine by adopting partial ciliary reversal (PaCR) swimming modes. At threshold concentrations (1-3 μM), the cells initially showed no response and continued in FLS. However, over a period of 10 to 15 min, an increasing percentage of the cells adopted a looping motion type with the loops being approximately 20 body lengths in diameter (i.e., very wide loops). There was a clear reduction in the frequency of spontaneous avoiding reactions since the cells, once having adopted a looping motion, generally did not show any avoiding reactions. After 15 to 20 min, the looping behavior slowly subsided and by 30 min, most cells had returned to FLS. At $\geqslant$ 8 μM quinine, most of the cells adopted a tigher circling motion (circle diameter of 5-10 body lengths) within 2 min of contact with the quinine-containing solutions. This behavior also slowly declined with time. At $\geqslant$ 32 μM, quinine induced a shock reaction followed within 1 min by periodic ciliary reversal (PCR) and later by a very tight circling motion (circle diameter of less than 5 body lengths). This behavior did not significantly change with time. At very high concentrations ($\geqslant$ 125 μM), the quinine was very toxic and within a few minutes, the cell motion slowed, body deformation

often occurred and occasionally cell lysis or blebbing was seen before the cells became immobilized — sometimes in a halo of discharged mucus (but not trichocysts). Shortly before immobilization, ciliary metachrony was clearly disturbed and the cells were characteristically shorter with granular cytoplasm and frequently with contractile vacuoles arrested in the dilated stage.

A number of experiments were carried out to assess if the toxic effects observed in the adaptation buffer containing 0.016 mM $CaCl_2$ could be a phototoxic effect. However, no obvious differences in cell motion (in the presence of all concentrations of quinine tested) were observed when the studies were carried out under (i) dark field illumination (fluence rate of $\sim$ 0.25 $W \cdot m^{-2}$), (ii) diffuse illumination (fluence rate of $\sim$ 0.9 $W \cdot m^{-2}$), (iii) under direct illumination with white light (fluence rate of $\sim$ 4 $W \cdot m^{-2}$) or (iv) under dim red light ($>$ 650 nm; incident fluence rate of $\sim$ 2.8 $W \cdot m^{-2}$). In addition, conventional assessment of the toxicity of quinine was carried out by exposing the cells, in adaptation buffer, to various concentrations (0.4, 0.9, 1.9, 3.9, .... 156 μM) of quinine over a 24 hour period. The $LD_{50}$ was the same, within experimental error, when such tests were carried out under laboratory fluorescent lighting or with the cell suspensions placed in a dark cupboard. The $LD_{50}$ was 2-4 μM. The cells, although immobilized, remained intact unless the quinine concentrations were $\geqslant$ 64 μM.

When adaptation buffers containing 0.031 or 0.062 mM $CaCl_2$ were used (i.,e. with the cells now adapted to a calcium level higher than in the hay infusion), direct exposure to quinine elicited threshold effects at $\sim$ 8 and 32 μM respectively and the circling motions induced were only seen at $\geqslant$ 32 μM and only after a delay of several minutes. Shock reactions (followed by PCR and then circling) was seen at $\geqslant$ 64 μM in the presence of 0.031 mM $CaCl_2$ and only at 0.125 mM in the presence of 0.062 mM $CaCl_2$. At 0.031 mM $CaCl_2$, immobilization occurred at 0.125 mM quinine but only after 10 to 15 min. Immobilization occurred at $\geqslant$ 0.25 mM quinine with 0.062 mM $CaCl_2$ (but at rather variable times ranging from 5 to 30 min). The relative sensitivity to quinine is thus inversely related to the calcium levels in the solution. Since the culture medium contained $\leqslant$ 0.05 mM total $CaCl_2$, most of the following studies were carried out at 0.016 or 0.031 mM $CaCl_2$.

### Effect of Quinine on the Responses of *Paramecium* to KCl

*Paramecium* responds to stimulation with 8 mM KCl by immediately shifitng into a continuous ciliary reversal (CCR) mode which is followed by cell gyration, cell spinning, cell looping and finally FLS is regained.

A full description of these responses for cells adapted to and tested in the presence of 0.016 mM $CaCl_2$ is provided elsewhere (Doughty 1986). The response sequence is, however, qualitatively the same if the cells are adapted to 0.016, 0.031 or 0.062 mM $CaCl_2$ and then tested in the presence of calcium over the entire range of 0.0039 mM to 0.5 mM. Previous studies by a number of investigators (Grębecki 1964, 1965, Kamada and Kinosita 1940, Kuźnicki 1966, Naitoh 1968, Doughty and Dodd 1978) show that the range of calcium concentrations, at which this response is shown to KCl, extends over a wider range — providing higher concentrations of KCl are used at calcium levels above 1 mM. In agreement with previous studies (Grębecki 1964, Naitoh 1968), it is found that the response to KCl is greatest (as assessed by the duration of either the CCR sequence or the total time the cells spend in reversed swimming before return to FLS, $t_R$) at calcium levels of 0.016 to 0.062 mM (Fig. 2 and 3). The peak response

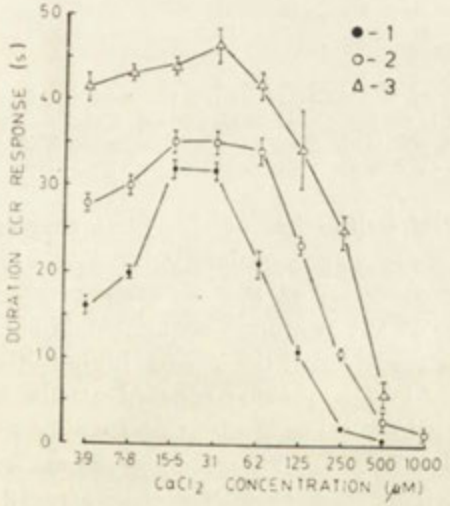


Fig. 2. Duration of the continuous ciliary reversal (CCR) response induced by 8 mM KCl in the presence of different total $CaCl_2$ levels in the test solution. Results are means ± SEM for 10-12 determinations under each condition. Cells were adapted to 1 — 0.016 mM $CaCl_2$ 2 — 0.031 mM $CaCl_2$ and 3 — 0.062 mM $CaCl_2$

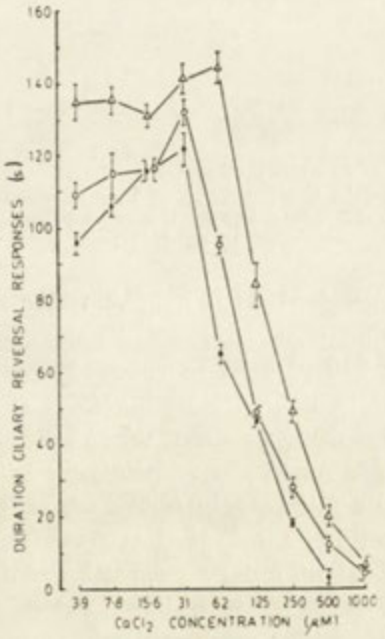


Fig. 3. Duration of the total period of ciliary reversal ($t_R$) iduced by transfer of cells to 8 mM KCl in the presence of different concentrations of $CaCl_2$. Details and symbols as Fig. 2

occurs at slightly different calcium concentrations depending on the level to which the cells were adapted (see also Kamada and Kinosita 1940). At higher calcium concentrations in the test solutions, the res-

ponses get progressively shorter in duration until, at 0.5 mM $CaCl_2$, no response is elicited by stimulation with 8 mM KCl.

If the cells are simultaneously presented with an 8 mM KCl stimulus in the presence of different concentrations of quinine in adaptation buffer containing 0.016 or 0.031 mM $CaCl_2$, the quinine clearly inhibits the KCl-induced CCR response (Fig. 4). The inhibition is concentration-

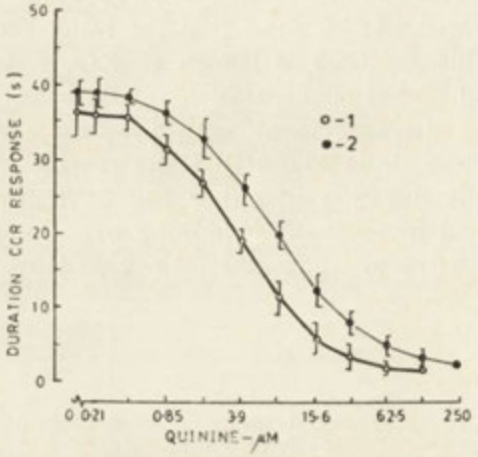


Fig. 4. Effect of quinine on the duration of the CCR response induced by 8 mM KCl in the presence of 1 — 0.016 mM $CaCl_2$ or 2 — 0.031 mM Ca $Cl_2$. Cells adapted to the same calcium level as in the test solution. Results are means ± SEM of 15-19 determinations under each quinine concentration

dependent with a 50% reduction in the duration of the CCR response being observed at 4-8 μM. The response was completely blocked at 62 or 250 μM. In marked contrast, the duration of the recovery phase of the response following CCR (i.e., the PaCR response sequence) is significantly increased when the quinine concentrations are higher than 4 μM or 16 μM respectively (Fig. 5). At high quinine concentrations, the cells fail to return to FLS — staying in a wide circling or looping motion for most of the time with only very occasional spontaneous and transient ciliary reversal events. Such cells were still motile after 2 h of exposure to quinine but $\simeq$ 75% of the cells were immotile by 12 h. Those that were still swimming were sluggish and also exibited periods of non-progressive motion (turning slowly only).

Preincubation of the cells with either 2, 4, 8 or 16 μM quinine prior to stimulation with 8 mM KCl (without any added quinine in these test solutions) essentially produced little lasting effect in that partial inhibition of CCR (Fig. 6) or augmentation of $t_R$ (data not shown) was only observed if the cells were preincubated in the higher concentrations of

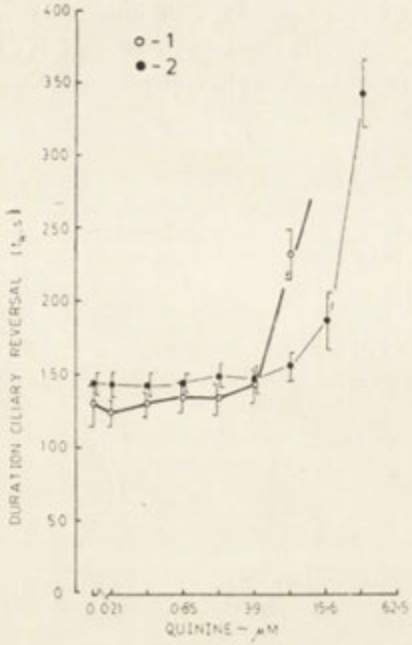


Fig. 5. Effect of quinine on the duration of the total period of ciliary reversal ($t_R$) induced by 8 mM KCl in the presence of 1 — 0.016 mM $CaCl_2$ or 2 — 0.031 mM $CaCl_2$. Other details as Fig. 4

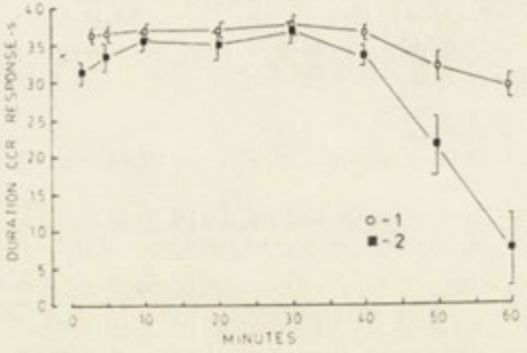


Fig. 6. Effect of various times of preincubation of cells in quinine-containing adaptation buffer (0.016 mM $CaCl_2$) on the duration of the CCR response subsequently induced by transfer of cells to an 8 mM KCl test solution in the absence of quinine. Results are means ± ± SEM for 8 - 11 determinations at each time period. 1—8 μM quinine, 2—16 μM quinine. No effect was observed at lower concentrations of quinine

quinine for over 40 min. The quinine thus reversibly interacts with the cells in order to produce alteration of the KCl-induced response.

The antagonistic effect of quinine was very dependent upon the extracellular calcium concentration. Testing of the cells (adapted to either 0.016 mM or 0.032 mM $CaCl_2$) in the presence of various test concentrations of $CaCl_2$ and either 2, 4, 16 or 32 μM quinine, showed that the inhibitory effect was greatest at lower extracellular $CaCl_2$ levels when the CCR response duration was assessed. At $CaCl_2$ levels higher than 0.125 mM, only partial inhibition was observed (Fig. 7): in the presence of 0.25 mM $CaCl_2$, then a partial inhibition the small residual

CCR response was observed with 125 μM quinine. At 0.25 mM $CaCl_2$ and with the use of 16 mM KCl as the stimulus, similar partial inhibition (45%) was also observed. Further studies were not pursued at these

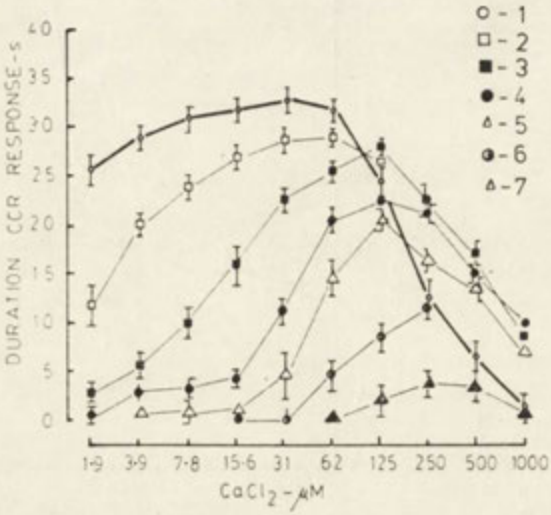


Fig. 7. Duration of the CCR responses induced by 8 mM KCl in the presence of various concentrations of $CaCl_2$ in the additional presence of various concentrations of quinine added to the test solutions. Cells adapted to 0.016 mM $CaCl_2$ and then transferred to test solutions containing the indicated $CaCl_2$ levels. Results are mean ± SEM for 10 - 12 determinations under each condition. 1 — control cells, and then the following concentrations of quinine: 2 — 2 μM, 3 — 4 μM, 4 — 8 μM, 5 — 16 μM, 6 — 32 μM and 7 — 62 μM

levels for, even with the presence of 0.5 mM $CaCl_2$ (with or without adaptation), the quinine plus KCl treatment always resulted in immobilization of the cells within a few minutes despite the fact that the cells could tolerate either chemical alone for extended periods.

## Effect of Quinine on the Responses of *Paramecium* to NaCl

In the presence of 0.016 mM $CaCl_2$, stimulation of *Paramecium* with 8 mM NaCl elicits a periodic ciliary reversal. The cells were carefully monitored with the event marker for the first 60 s after stimulation with NaCl so that both the duration of the initial PCR event and that of successive events could be assessed. At this $CaCl_2$ concentration (and at 0.031, 0.062 and 0.125 mM), the cells respond within less than a second with a short backward swimming event and then generally switch into a response pattern in which a sequence of transient ciliary reversals ("avoiding reactions") are occasionally punctuated by longer duration backward swimming periods similar in duration to the initial event (for an example see Fig. 8). The duration of the initial PCR event was found to be 1.59 ± 0.23 s (n = 27). This was not significantly different from the average duration of the subsequent PCR events that occurred over the first 60 s after NaCl (duration = 1.63 ± 0.12 s, n = 186). While it must be acknowledged that there is substantial variance in the duration of these events (Fig. 9), the overall frequency of directional changes

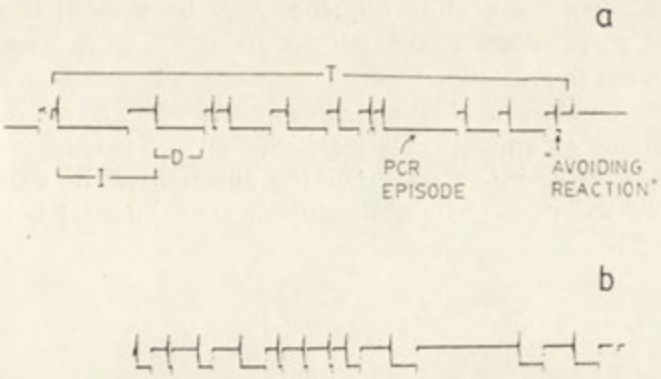


Fig. 8. Typical event marker print out trace showing the occurrence of avoiding reactions and PCR episodes for cells stimulated with 8 mM NaCl. (a) control cells. I — interval between ciliary reversal events, D — duration of PCR event. (b) cells in the additional presence of 32 μM quinine showing the reduced duration of the PCR episodes

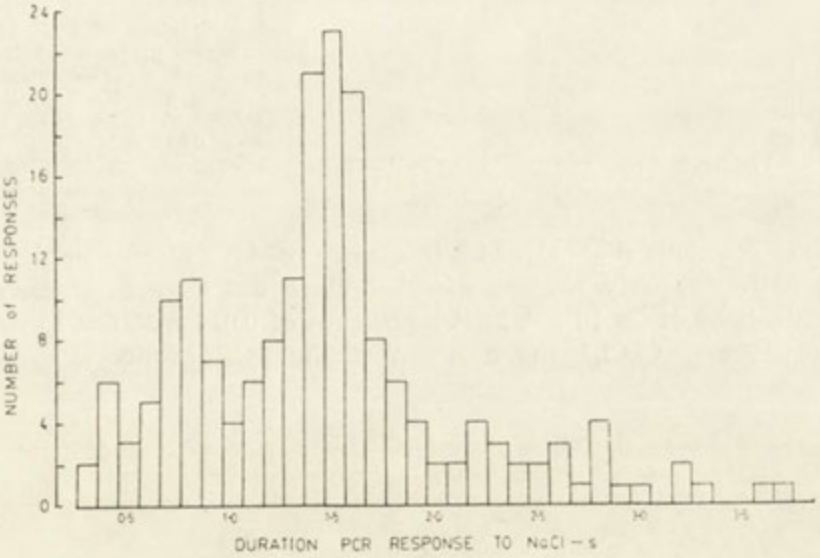


Fig. 9. Histogram showing the durations of the PCR response episodes given in the first 60 s after transfer to a test solution containing 8 mM NaCl in the presence of 0.016 mM $CaCl_2$

(with or without longer periods of backward swimming) over the first 60 s was 53 ± 2 directional changes/min (27 paths analyzed). The mean interval between all responses (i.e., AR's was 1.07 ± 0.04 s, (n = 342). Similar results were obtained for cells adapted to and tested in the presence of 0.031 mM $CaCl_2$ (see below). After 60 s of exposure to 8 mM NaCl, the frequency of the PCR response declines slowly over the next

15 min. A full analysis of this sequence will be presented elsewhere (manuscript in preparation) since such details of the PCR response have not been analyzed previously.

In adaptation buffer containing 0.016 mM or 0.031 mM $CaCl_2$, the low concentrations of quinine that markedly attenuated the response to KCl had essentially no effect on either the duration of the PCR responses (Fig. 10) or the (independently-assessed) time interval between the

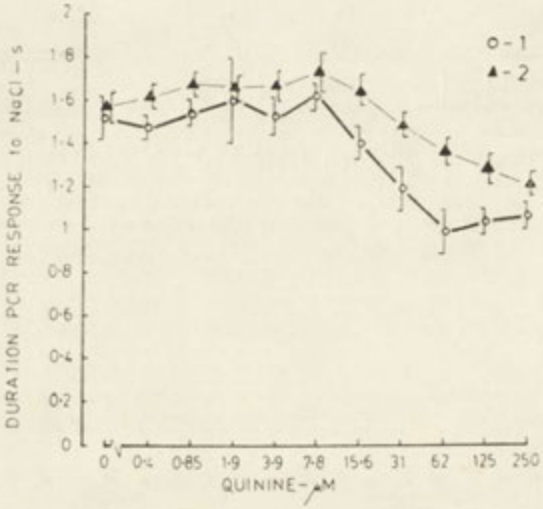


Fig. 10. Effect of quinine on the duration of PCR response episodes given by cells in the first 60 s after stimulation with 8 mM NaCl in the presence of 1 — 0.016 mM or 2 — 0.031 mM $CaCl_2$. Results are means ± SEM for 12-26 episodes recorded under each condition

avoiding reactions (Fig. 11). The frequency of AR's in any single swimming path was only slightly affected (data not shown). However, at concentrations of $\geqslant$ 16 μM in the presence of 0.016 mM $CaCl_2$) or $\geqslant$ 32 μM (0.031 mM $CaCl_2$), the duration of the PCR events was reduced

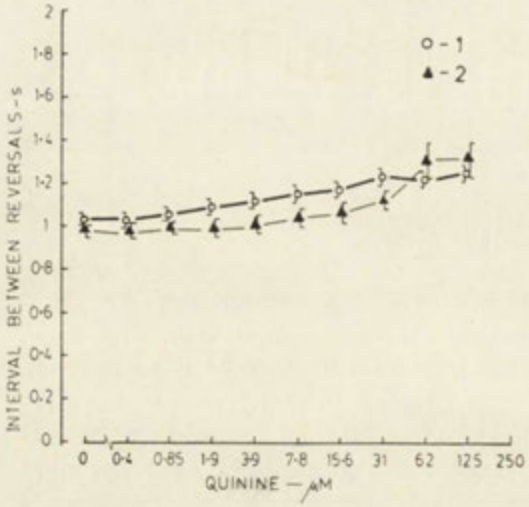


Fig. 11. Effect of quinine on the interval between PCR episodes or avoiding reactions during the first 60 s after stimulation of cells with 8 mM KCl in the presence of 1 — 0.016 mM or 2 — 0.031 mM $CaCl_2$. Results are means ± SEM for 126-184 interval times for each condition

slightly (Fig. 10) and the interval between all responses (AR's and PCR episodes) increased slightly (Fig. 11). While a slight decrease in the frequency of AR's was seen with the higher concentrations of quinine (especially with testing in the presence of 0.031 mM $CaCl_2$), part of the reduction in this frequency can readily be attributed to an obviously slower swimming velocity of the cells. If such a slowing of forward swimming speed also is applied to backward swimming (i.e., during the PCR episodes), then the inhibitory effects measured will be smaller than actually occurring.

### Effect of Quinine on the Responses of *Paramecium* to $BaCl_2$

When transferred into a solution containing $BaCl_2$ and lower concentrations of $CaCl_2$ (0.016 or 0.031 mM), the cells respond immediately by switching into a PCR-type behavior. This PCR-type behavior differs from that given to an NaCl stimulus in that the response does not obviously decrease in intensity (frequency) or duration (of the individual responses) until that point in time, 20 to 30 min after contact, when the $BaCl_2$ exerts a toxic effect. The cells' swimming speed then slows (sometimes fairly rapidly) and immobilization occurs. In addition, there are only occasional avoiding reactions (i.e., responses of $\leqslant$ 0.1 s).

To facilitate comparison with the studies on NaCl, only the initial response and those responses recorded over the first 60 s of contact will be considered. For cells transferred into 0.125 mM $BaCl_2$ in the presence of 0.016 mM $CaCl_2$, the initial response averaged 1.42 $\pm$ 0.17 s (n = 34) while the average of all responses recorded over 60 s was 1.69 $\pm$ 0.06 s (n = 226). As with the responses to NaCl, there is considerable hetero-

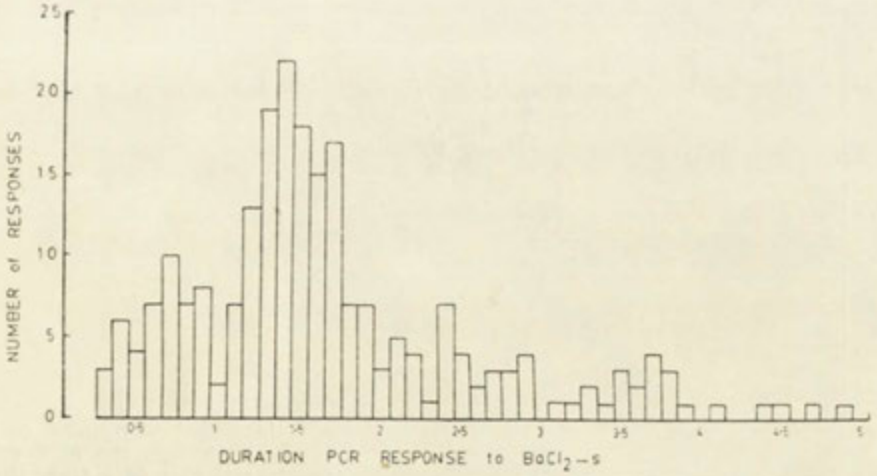


Fig. 12. Histogram showing the duration of the PCR episodes given by cells in the first 60 s after stimulation with 0.125 mM $BaCl_2$ in the presence of 0.016 mM $CaCl_2$

geneity in the response durations (Fig. 12). The mean interval time was 2.21 ± 0.09 s (n = 226). For cells transferred into 0.25 mM $BaCl_2$ in the presence of 0.031 mM $CaCl_2$, the initial response duration was 1.64 ± 0.11 s (n = 17) while the average response duration over 60 s was 1.84 ± 0.04 s (n = 92). The mean interval time was 1.96 ± 0.11 s.

At both $CaCl_2$ concentrations, quinine had small effects on the responses to $BaCl_2$. Threshold effects were detectable at 4 μM in that both the

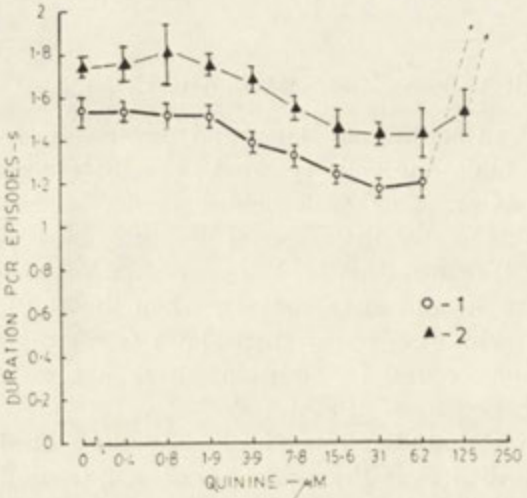


Fig. 13. Effect of quinine on the duration of PCR episodes given by cells in the first 60 s after stimulation with 0.125 mM $BaCl_2$ in the presence of 0.016 mM $CaCl_2$ (1) or 0.25 mM $BaCl_2$ in the presence of 0.031 mM $CaCl_2$ (2). Results are means ± SEM for 63-89 episodes under each condition

duration of the PCR events became slightly shorter (Fig. 13) and the interval between the events became slightly shorter at quinine concentrations of ⩾ 7.8 μM (Fig. 14). While the mean durations of the PCR

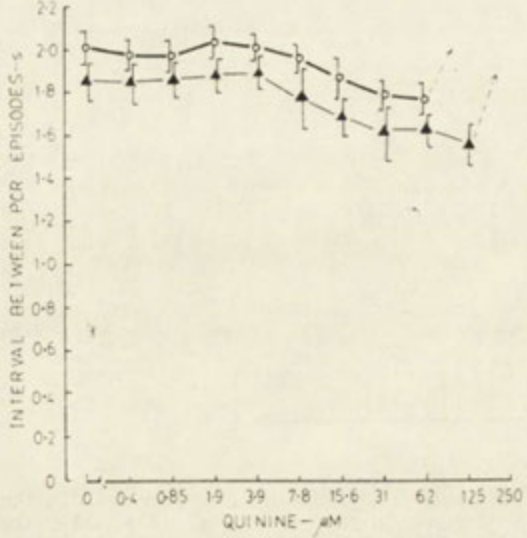


Fig. 14 Effect of quinine on the interval between the PCR episodes in the first 60 s after stimulation of cells with $BaCl_2$. Other details as Fig. 13

episodes given to a $BaCl_2$ stimulus only change slighty, a closer analysis indicates that the action of quinine on the $BaCl_2$ response is more complex than this. As illustrated in Fig. 15, a histogram of the response durations observed following stimulation of the cells by 0.125 mM $BaCl_2$ in the presence of 0.016 mM $CaCl_2$ suggests two discrete PCR

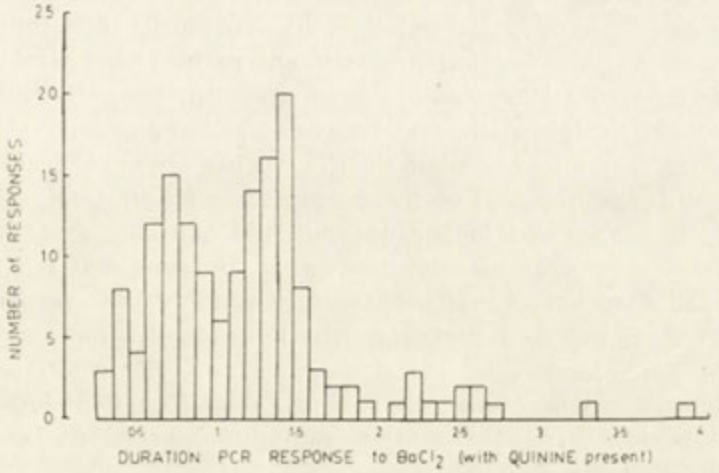


Fig. 15. Histogram showing the duration of PCR episodes given by cells in the first 60 s after stimulation with 0.125 mM $BaCl_2$ in the presence of 0.016 mM $CaCl_2$ and 62 μM quinine

episode times of ~ 0.7 s and ~ 1.4 s in the presence of 62 μM quinine. An indication of this dual response of the cells can also been in Fig. 12.

To allow comparison with other studies on the $BaCl_2$-induced ciliary reversal behaviors, it can be noted that the frequency of the PCR response (when assessed independently by simply counting the number of reversals in any swimming track over a period of approx 60 s e.g., see Yarbrough and O'Kelley 1962), increases slightly as the quinine concentration is raised. Thus, for example, while control cells show a frequency of PCR episodes of ~ 20/min, cells treated with $BaCl_2$ in the presence of 62 μM quinine show a frequency of ~ 30 episodes/min.

Alternatively, the $BaCl_2$-induced responses can be assessed as the time that the cells actually spend in backward (as opposed to forward) swimming (Ling and Kung 1980). Analysis of the swimming tracks shows, however, that both control cells and those treated with $BaCl_2$ in the presence of up to 62 μM quinine spent 72-74% of their time swimming backwards. In other words, the quinine modifies the timing of the $BaCl_2$-induced response — both the duration of the individual episodes and the interval between the responses.

In contrast, higher concentrations of quinine (i.,e $\geqslant$ 62 μM) had a very

different action on the $BaCl_2$-treated cells. Despite the concentration-dependent reduction in the duration of the PCR response by quinine, the higher concentrations shifted the cells into a longer duration CCR-type response (instead of PCR). This was not recorded in any detail since preliminary evaluation revealed no obvious pattern. However, such observations indicated that the cells would first show short duration PCR responses over 3 to 7 cycles and then spontaneously shift into a longer duration backward swimming in a straight path. This first longer response was 5 to 10 s long. After a brief gyration, the cells then usually again resumed straight path, fast backward swimming but with this second episode being longer than the first. This sequence was generally repeated with the duration of the backward swimming episodes getting progressively longer and longer until, in the extreme, some cells were observed to swim continuously backwards for over 300 s. The initial analyses failed to detect and defined summation of the response durations with each repeated cycle, i.e., the duration did not increase in a defined way but was very variable and some cells could later show a very long duration event followed by a shorter CCR-type episode. Transient periods of FLS were occasionally observed in between the backward swimming episodes.

## Discussion

Quinine is clearly able to prevent the induction of continuous ciliary reversal by KCl stimulation of the cells. Quinine thus represents a new antagonist of KCl-induced ciliary reversal. The relative potency of quinine decreases as the extracellular $CaCl_2$ levels are raised so that higher concentrations of quinine are needed to attenuate the response at higher calcium levels. In contrast, quinine has more subtle effects on the responses to NaCl and $BaCl_2$ and does not act as a simple antagonist (at least under the range of conditions tested). In fact, at the higher concentrations, quinine markedly augments the responses to $BaCl_2$.

The mechanism of action of quinine is unclear at this time. However, several of the observations made here and studies on other systems indicate a specific action that warrants further investigation. In addressing the action and effects of quinine on *Paramecium*, several questions can be asked.

Firstly, is the blockade of the $K^+$-induced CCR response merely a toxic effect on the cells? The results here indicate that although the alkaloid is toxic towards these cells, the state of the cells when the $K^+$-CCR is inhibited does not suggest severe toxicity. Unquestionably, and in agreement with earlier studies (see introduction), quinine (and

related compounds) are toxic to the cells when exposed over prolonged periods. However, such toxic effects are clearly evident in the cells with changes in both the cell cytoplasm and cell shape being observed. In addition, acute exposure of the cells to higher concentrations of the alkaloid also produced cytotoxic effects. The slowing of cell motion, the body deformation and cell blebbing are similar to those reported for *Paramecium* exposed to many other toxic agents (Kalmus 1931, Wichterman 1953). Such cytotoxic effects are clearly reduced by elevation of extracellular calcium levels in the adaptation/test solutions. Such calcium-related attenuation of cytotoxicity has also been reported for cells exposed to nickel salts (Andrivon 1972), ferric salts (Sokoloff 1923), acids (Collett 1919) and to elevated temperatures (Chalkley 1930). Such actions of calcium must therefore be considered as a general, non-specific protective action but which highlights the importance of calcium ions in the physiology of these cells. It is possible therefore that the toxicity observed following chronic exposure (in culture medium) may be in part due to the low levels of total calcium in these hay-cerophyll infusions. It can be noted, however, that although other cerophyll cultures have been reported to contain only 10 μM total calcium (Browning and Nelson 1976), adequate growth of *Paramecium* (presumably in an axenic medium) has been reported at $\leqslant$ 50 μM total calcium (Van Wagtendonk 1974). Bacterized hay infusions are not normally supplemented with calcium salts so contain only that calcium derived from the water used and the vegetative matter used to prepare the infusion. Indeed, cultures have been prepared from a single grain of wheat in 100 ml of distilled water (Huber et al. 1974). Chalkley's medium (once often used in protozoan studies), is formulated to contain only 0.057 mM calcium (Chalkley 1930). It should be noted that the cultures used in the present study were prepared for use on a discontinuous basis in that serial subculturing from late-logarithmic cultures was employed with the fully-grown cultures being used for experiments rather than being replenished. Such is a very different scenario from maintaining a large stock culture (from which cell samples can be periodically taken) which is occasionally replenished with additional organic matter and fluid as growth characteristics dictate. Such can clearly result in a progressive elevation in total calcium to nearly millimolar levels (Bancroft 1906). As a final support of the culture and experimental conditions used in this study, it can be noted that fresh natural water total calcium levels as low as 0.004 mM (16 mg/l) have been reported and values in the vicinity of 0.020 mM equivalents have been fairly widely reported (Armstrong and Schindler 1971, Carter et al. 1986, Keslo et al. 1986, Wetzel 1983). The total calcium con-

tent will of course depend on the hardness of the water supply (which will in turn play a dominant role in determining the pH of that natural water supply and thus the levels of ionized calcium: Plummer and Busenberg 1982). Only limited data appear to be available on the natural habitat of *Paramecium* (Armstrong and Schindler 1971, Nyberg and Bishop 1983).

A second and related question, that needs to be addressed within the context of this marked action of quinine on *Paramecium*, relates to the physiological health (or integrity) of the cells. The cells have been purposely studied in a medium that contains only modest levels of total calcium (0.016 to 0.062 mM). For what appear to be poorly defined reasons, *Paramecium* have traditionally been studied in media containing somewhat higher levels of total calcium (e.g., 0.5 to 1 mM: see Doughty and Dryl 1981 for commentary). Therefore, how much consideration must be given to an argument that the inhibitory effect of quinine is only observed because the cells are already under stress as a result of being suspended in a medium with only modest calcium levels? As indicated above, *Paramecium* very much appear to be able to tolerate these modest calcium levels for extended periods of time and it seems very plausible that the same modest levels have been used in other cases where hay infusions are used — especially on a discontinuous basis. Similarly, *Paramecium* can be also adapted to far higher calcium levels (Genermont 1969). The solutions used in the present study contain $Na^+$ and buffer as the only added species in addition to $CaCl_2$ but, at the modest $CaCl_2$ levels used, the cells exibited coordinated and robust motion for extended periods of time (Doughty 1986) in addition to active contactile vacuole activity (unpublished results). Requirements for extracellular $K^+$ have not been defined for *Paramecium* to my knowledge and it is acknowledged that the present solutions are deficient in $Mg^{2+}$ (that can be expected in the natural habitat along with $Ca^{2+}$). The present media are also possibly deficient in anions such as sulfate, phosphate and bicarbonate although the cells, in time, will be expected to generate sufficient $CO_2$ from respiration to add small ammounts of dissolved $CO_2$ (bicarbonate) into their extracellular medium. The excretion of other ions and metabolites, in trace ammounts, can also be expected with higher cell densities. In defense of the use of the present solutions, other workers have successfully adapted *Paramecium* to 0.01 mM $CaCl_2$ (in the absence of added KCl: Grębecki 1965) or 0.05 mM $CaCl_2$ has been used in a variety of studies (Yamaguchi 1963, Kuźnicki 1966, Schultz et al. 1986). Even allowing for substantial improvements in the quality of distilled water over the years, it is noteworthy that the cells "usually were able to survive well" in

a solution of 0.24 mM $NaHCO_3$ (Akita 1941). In the present studies, the cells did not survive more than 30 min if placed in adaptation buffers to which no $CaCl_2$ had been added (i.e., total calcium $< 0.001$ mM). The calcium levels used in the present adaptation buffers are also clearly higher than those at which (high) concentrations of monovalent cation salts were reported to cause disruption (permeabilization) of the ciliary or cellular membranes (Hildebrand and Dryl 1983) and do not cause cellular immobilization except at extremes of pH (Doughty 1986). The present studies on the effects of extracellular calcium on the duration of $K^+$-ciliary reversal are qualitatively similar to those reported by others in that the maximum response is observed at low calcium levels and that further elevation in calcium attenuates the CCR response (Kamada and Kinosita 1940, Grębecki 1964, 1965, Kuźnicki 1966, Naitoh 1968). Lowering of extracellular calcium was reported (qualitatively) to reduce the duration of PCR responses to $BaCl_2$ (Eisenberg-Hamburg 1932, Kuźnicki 1966). Other studies (some at significantly higher overall ion levels) report that, as calcium is substantially lowered to (increasing) $BaCl_2$ levels, the cells show either longer duration responses (Kuźnicki 1966, Ling and Kung 1980) or a significantly higher frequency of responses (Yarbrough and O'Kelley 1962). The NaCl-induced response has been reported to be enhanced by lowering extracellular calcium levels (Kuźnicki 1966, Yarbrough and O'Kelley 1962). Thus, in the present study (where low concentrations of quinine were found to suppress totally $K^+$-induced CCR while only reducing the duration of the responses to $BaCl_2$ and NaCl) the effects are not obviously consistent with a general injurious response of the cells studied under subnormal calcium levels.

The third issue relates to the possible mechanism(s) of action of quinine. Quinine, as an organic base with a $pK_a$ 9.7 (Merck Index) (quinidine has a $pK_a$ of 8.6: Trung and Sirois 1987) will be almost completely ionized at pH 7.1. Thus, the levels of cationic quinine at pH 7.1 will be similar to the expected ionized levels of calcium when the higher concentrations of quinine are used at the lowest calcium levels. Thus, part of the quinine action under these conditions might be attributed to a simple displacement mechanism to remove surface $Ca^{2+}$ or screen surface $Ca^{2+}$ on the cells — thus attenuating the responses. The shift in the relative potency of quinine as a function of increasing extracellular calcium is consistent with this type of competitive action and the same has been suggested when quinine is used as a bitter taste ligand (Kumazawa et al. 1986). Quinine, in this latter case, was considered to be absorbed onto hydrophobic sites near the membrane surface rather than acting simply at the cell surface-solution interface (Kumazawa

et al. 1986). The lack of permanent effect of quinine (when cells are preincubated with quinine) is consistent with its being readily removed from the surface of the cells. Previous studies on the sensitivity of *Paramecium* to quinine as a chemorepellent also found that an elevation in extracellular calcium also resulted in higher threshold levels for quinine (Dryl and Kurdybacha 1978). Quinine does not appear to have any actions as a $Ca^{2+}$ chelator and is thus not simply acting to remove ionized calcium from the bulk solution.

The marked effects of quinine on the cellular renormalization (i.e., the return to FLS after induction of CCR) is also not obviously consistent with a simple deprivation of calcium from the cells since lowering calcium reduces the total response duration rather than markedly enhancing it. While further studies are obviously needed on the calcium sensitivity of the NaCl and $BaCl_2$-induced ciliary reversal responses, the high selectivity of low concentrations of quinine on these responses and the very marked enhancement of the $BaCl_2$-induced responses by higher concentrations of quinine (which by themselves do not induce ciliary reversal) is also not consistent with simple calcium effects resulting from deprivation of calcium in the bulk solution.

The action of quinine does not appear to be via a photo (toxic) effect — at least for acute exposure in test solutions for light had no obvious effect on either cell motility or behavioral responses in the presence of quinine. However, while quinine does not appear to have the chance to be taken up by the cells in order to suppress $K^+$-induced CCR, quinine may be taken up in the longer term. Here then it must be acknowledged that *Paramecium* (unpublished observations), like *Tetrahymena* (Ruben et al. 1982) contains protoporphyrin IX-like molecules (especially in stationary phase of growth — visualized as bright red fluorescence). Quinine can form a complex with such ferri-protoporphyrin compounds (Blauer 1986). Furthermore, high concentrations (100 μM) of quinine have been reported to inhibit cell division in *Tetrahymena* through suppression of DNA, RNA, protein and lipid synthesis (Conklin and Chou 1969, Conklin et al. 1970). Research on other systems suggests that general membrane perturbation by quinidine (Nakae and Asada 1986) and mefloquine (Chevli and Fitch 1982) can occur at millimolar concentrations when evaluated in high ionic strength media. Finally, high concentrations (100 μM) of quinine have been reported to act as inhibitors of mitochondrial $K^+$ permeability (Diwan 1986). However, the immediacy of its action on the behavioral responses of *Paramecium* is reasonable evidence against its action via such internal sites.

Thus, it is proposed that quinine has another action on *Parame-*

*cium* — an action that results in an attenuation of the normal cell renormalization processes after KCl stimulation and produces a markedly enhanced response to $BaCl_2$. Quinine has been widely reported as an antagonist of $K^+$ ion channels in a variety of cell systems (Fishman and Spector 1981, Grossman et al. 1981, Herman and Hartung 1982, Dixon et al. 1984, Findlay et al. 1985. Iwatsuki and Peterson 1985). For these systems, as with *Paramecium* (Naitoh et al. 1973, Machemer and Ogura 1979, Satow and Kung 1980, Saimi et al. 1983), it is uncertain as to how much such $K^+$-currents are normally regulated by membrane potential (i.e., are voltage -regulated) or by internal $Ca^{2+}$ (i.e., $Ca^{2+}$-activated $K^+$ channels). Thus, quinine may block voltage- or $Ca^{2+}$-activated $K^+$ channels. The blockade of outward $K^+$ currents in *Paramecium* by quinine would be expected to slow (or prevent) ciliary renormalization by preventing that aspect of membrane repolarization after the voltage-induced $Ca^{2+}$ influx. Quinine, in this respect, acts as $BaCl_2$ does in preventing activation of outward $K^+$ currents and thus, at high concentrations, can shift the membrane into a all-or-nothing electrogenesis (Van Houten 1979, Ling and Kung 1980). At sufficiently high levels of $Ba^{2+}$ (specifically, a high ratio of $Ba^{2+}$ to $Ca^{2+}$), the short bursts of all-or-nothing electrogenesis can be replaced by periods of sustained depolarization with ensuing long duration backward swimming (Ling and Kung 1980). The action of higher concentrations of quinine on the responses to $BaCl_2$ is consistent with quinine augmenting $K^+$ channel blockade produced by $BaCl_2$.

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# The Effect of Pretreatment of *Tetrahymena pyriformis* with Colistin on the Incorporation of this Antibiotic into the Cell Membrane

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*Synopsis.* The part played by the cell membrane of *Tetrahymena pyriformis* in the process of physiological adaptation effected by colistin was investigated. Incorporation of colistin into the lipid fragments of the cell membrane during pretreatment with colistin seems to be a mechanism responsible for blocking the colistin incorporation into the cell membrane. Absence of incorporation of labelled colistin into the cell membrane, was achieved only in the case of early-log phase cells. Lack of the effect in cells from other growth phases might be due to an increase in the lipid content of *Tetrahymena* cell membrane observed with ageing of the culture. The "receptor-like" membrane pattern in *Tetrahymena* appears to play a less significant part in the process of cell adaptation to colistin, although such a mechanism cannot be entirely excluded.

Adaptation of cells to environmental changes is observed in a variety of organisms, such as animals (Brinkley et al. 1967, Carlson and Suttie 1967), plants (Grover and Moore 1961, Gundersen and Wadstein 1962) and protozoa (Frankel 1970).

One of the ways by which organisms adapt to the continuous presence of non-lethal concentrations of a cell metabolism inhibitor is the so-called physiological, or phenotype adaptation. Although this type of adaptation has been described in detail, especially as observed in *Tetrahymena* (Frankel 1970, Szablewski 1984, 1985), the underlying mechanism of the phenomenon remains unknown. The findings by other authors allow to conclude that the process consists of three stages: (1)

alterations in the cell membrane permeability permitting penetration of more particles of the substance into the cell (Roberts and Orias 1974), (2) deactivation of the inhibitor inside the cell (Roberts and Orias 1974, Stubblefield 1964), (3) removal of the inhibitor from the cell (Frankel 1970, Heyer and Frankel 1971). Although very little is known of each of the stages of physiological adaptation, the role played by the cell membrane in the process is so far the least recognized element.

It is likely that alterations in permeability of the cell membrane in the course of physiological adaptation are produced by one (or more) of the following mechanisms: (1) saturation of the cell membrane with the inhibitor; this type of response would concern essentially these metabolism inhibitors which do not penetrate into the cytoplasm and act on the cell membrane (e.g. colistin); (2) saturation of the cell membrane receptor sites with the inhibitor; such a reaction would occur with both toxins entering the cell (e.g., cycloheximide) and non-penetrating agents; (3) blockade of the toxin receptor sites and/or transport system into the cell; this reaction would partly result from the previous reaction and take place in the case of inhibitors penetrating into the cell.

The aim of the present study was to investigate the first of the mechanism listed, i.e., to check whether pretreatment of cells with the inhibitor for a given period of time would prevent incorporation of the substance into the *Tetrahymena* cell membrane when it was again added to the culture. For this purpose the polypeptide antibiotic colistin and colistin labelled with dansyl chloride were employed in the study.

## Materials and Methods

### The Cells

The experiments were carried out on an amicronucleate strain of the ciliate *Tetrahymena pyriformis* GL. The cells were cultivated in Erlenmayer flasks containing 25 ml of the medium (1.5% proteose-peptone and 0.1% yeast extract — Difco) at 28°C.

### Preparation of the Complex Colistin-Fluorescent Compound (ColDC)

A fluorescent derivative of colistin (ColDC) was prepared by coupling 1-dimethylaminonaphtalene-5 sulphonyl chloride (dansyl chloride) with γ-amino group of αγ-diaminobutyric acid radicals in the polymyxin molecule. Colistin was labelled according to the partially modified method originally proposed by Newton (1955) (Szablewski — in prep.).

## The Effect of Pretreatment of *Tetrahymena* with Colistin or ColDC upon the Process of Phagocytosis

The aim of the experiments was to investigate whether the so-called "cross-adaptation" effected by colistin or ColDC could occur in *Tetrahymena*.

The experiments were carried out on cells derived from the early log growth phase, late log phase and the stationary growth phase. The conditions of *Tetrahymena* pretreatment and substances added after 24-h pretreatment with colistin or ColDC are presented in Table 1.

Table 1

Conditions of pretreatment of *Tetrahymena* and chemicals added to particular cultures after 24-h pretreatment

| Number of culture | Pretreatment with: | After 24-h pretreatment added: |
|---|---|---|
| 1 | Colistin 0.1 mM | Colistin 0.1 mM |
| 2 | Colistin 0.1 mM | ColDC 0.1 mM |
| 3 | ColDC 0.1 mM | ColDC 0.1 mM |
| 4 | — | ColDC 0.2 mM |
| 5 | — | — |

The phagocytosis test was performed in cups. 0.1 ml of ink suspension, prepared by dissolving 0.15 ml of ink in 4 ml of distilled water were added to 1 ml of cell culture.The cells were exposed to the ink suspension for 15 min at 28°C. Next, particular samples were fixed in formalin solution and the number of food vacuoles was counted in 50 cells.

## Long-lasting Pretreatment of *Tetrahymena* with Colistin

The experiments were carried out on cells from early log phase, late log phase and the stationary phase. Colistin at concentrations of 0.5 mM, 1 mM, 2 mM, 4 mM, 8 mM, and 16 mM was added to particular cultures. The seventh culture was control and therefore no antibiotic was added. After 24 h of the cell exposure to colistin, ColDC at a concentration of 0.1 mM was added to each culture and it was observed whether fluorescence of the cell mebrane and food vacuole membrane occurred.

The continous presence of 16 mM colistin in a culture is the highest concentration at which a fraction of cells can survive (Szablewski 1981). On the other hand, the antibiotic added to the culture at a concentration of 0.5 mM, although producing morphophysiological disorders in *Tetrahymena*, does not eliminate any cell from the culture (Szablewski 1984, 1985).

## Short-lasting Pretreatment of *Tetrahymena* with Colistin

The experiments were carried out exclusively on cultures derived from the early log phase. The antibiotic concentrations of 8 mM and 16 mM were used. After 30 min 0.1 mM ColDC was added to each culture and fluorescence of the cell membrane and food vacuole membrane was observed.

## Microscopical Observations

The samples were examined using a Carl Zeiss Amplival fluorescence microscope. An XBO 50 W lamp was used as a light source. UG 1(1.5) + BG 12 were excitation filters and OG 4 served as a barrier filter.

## Results

### The Effect of Pretreatment of *Tetrahymena* with Colistin or ColDC upon the Process of Phagocytosis

There is no difference between effects of colistin and ColDC on *Tetrahymena* cells while sensitivity of *Tetrahymena* to chemicals varies according to the phase of culture growth (Fig. 1).

In the case of control (Culture No. 5, see Table 1), the percentage of non-phagocyting cells ranged from 10 to 13 according to the growth

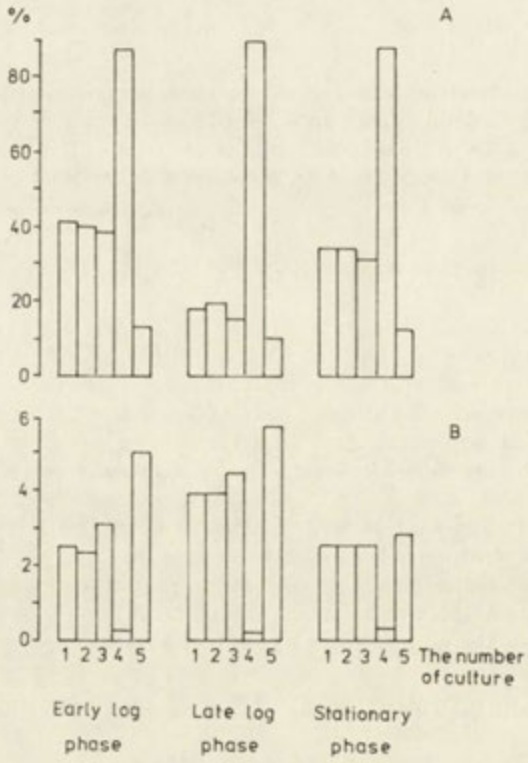


Fig. 1. Relationship between the phagocytosis rate and the conditions of pretreatment as well as culture growth phase. A — ordinate — per cent of non-phagocyting cells, B — ordinate — average number of food vacuoles formed per cell during 15-min exposure

phase culture, while the average number of food vacuoles in a cell varied from 2.8 in the stationary phase to 5.7 in the log phase. If 0.2 mM ColDC was added to the culture without previous pretreatment with any of the chemicals investigated (Culture No. 4, see Table 1), the rate of phagocytosis was found to decrease markedly. In this case the percentage of non-phagocyting cells varied from 87 to 89, whereas the average number of food vacuoles in a cell ranged from 0.2 to 0.3 (Fig. 1). With pretreatment of cells in the presence of 0.1 mM colistin or 0.1 mM ColDC and subsequent addition to the culture of colistin or ColDC in different combinations (Cultures No. 1, 2, 3, see Table 1) the results from those obtained in control cells. The phagocytosis rate was lower in the control culture (Culture No. 5, see Table 1), but higher in the culture, to which 0.2 mM ColDC was added without its earlier pretreatment with any of the chemicals investigated (Culture No. 4, see Table 1).

## The Effect of Long-lasting Pretreatment of *Tetrahymena* with Colistin upon Incorporation of ColDC into the Cell Membrane

Our results demonstrate that cell sensitivity to pretreatment with colistin differs according to the phase of culture growth (Table 2). The differences consist in possibility of blocking ColDC incorporation into

Table 2

The effect of *Tetrahymena* pretreatment with colistin at different concentrations upon fluorescence of the cell membrane and food vacuole membrane produced by ColDC, according to the growth phase and colistin concentration during pretreatment

| 2 \ 1 | Control | 0.5 mM | 1 mM | 2 mM | 4 mM | 8 mM | 16 mM |
|---|---|---|---|---|---|---|---|
| Early log | A, W | A, W | A, W | A, W | A, W | B, W | C, W |
| Late log | A, W | A, R | A, R | A, R | A, R | A, R | A, R |
| Stationary | A, W | A, R | A, R | A, R | A, R | A, R | A, R |

1 – Colistin concentration in the culture during pretreatment, 2 – Growth phase, A – Fluorescence of the cell membrane, food vacuole membrane and the posterior part of the cell in the cytopyge area, B – Very weak fluorescence of the cell membrane and food vacuole membrane and cytopyge area, C – Absent fluorescence of the cell membrane and food vacuole membrane. Evident fluorescence confined to the posterior part of the cell in the cytopyge area, W – White fluorescence, R – Red fluorescence.

the cell membrane on the one hand, and dissimilarities in the colour of fluorescence on the other hand. Thus, in cells derived from the early log phase, incubated for 24 h in the presence of 16 mM colistin, addition of 0.1 mM ColDC produced no fluorescence in any of the organelles. In the case of the culture incubated for 24 h in 8 mM colistin, the fluorescence of the cell membrane and food vacuole membrane was found to decrease markedly in intensity upon addition to the culture

of 0.1 mM ColDC as compared to the control and to the cultures, in which lower concentrations of colistin had been employed during pretreatment. However, pretreatment of *Tetrahymena* with the other concentrations of the antibiotic, i.e., 0.5 mM, 1 mM, 2 mM, and 4 mM did not produce visible changes in the fluorescence of the cell membrane and food vacuole membrane effected by ColDC, as compared to the control. In all samples, in which fluorescence of these organelles was observed, the cell membrane and food vacuole membrane revealed white fluorescence in the living cells, while in the dead cells white fluorescence was demonstrated in the whole cytoplasm (Table 2). Different effects were observed in the late log phase cells. In this case no blockade of the *Tetrahymena* cell membrane to ColDC was found as a result of the ciliates' pretreatment with all colistin concentrations studied. Upon addition to the culture of 0.1 mM ColDC, evident fluorescence of the cell membrane and food vacuole membrane was observed in all samples. However, in the *Tetrahymena* derived from this growth phase, living control cells demonstrated white fluorescence of the cell membrane and food vacuole membrane, in the living cells incubated for 24 h in the presence of all antibiotic concentrations studied the organelles were found to fluorescence red, while in the dead cells white fluorescence of the whole cytoplasm was observed. Additionally, in some living cells subjected to pretreatment with the antibiotic white fluorescing spots were seen in the posterior part of the ciliate cell (cytopyge?), although the cell membrane fluoresced red (Table 2).

The findings in the *Tetrahymena* derived from the stationary phase of culture growth were the same as in the late log phase cells. In this case, no effect of pretreatment with colistin upon ColDC incorporation into the *Tetrahymena* cell membrane was established (Table 2).

## The Effect of Short-lasting Pretreatment of *Tetrahymena* with Colistin upon ColDC Incorporation into the Cell Membrane

Short-term exposure of *Tetrahymena* to colistin at a concentration of 8 mM did not produce evident changes in the ColDC potential to incorporate into the cell membrane. In this case, as in the cells derived from the control obvious fluorescence of the cell membrane, food vacuole membrane and cytopyge effected by ColDC was observed. On the other hand, 30-min exposure to the antibiotic at a concentration of 16 mM produced a visible response in the cells. On addition of ColDC to the culture fluorescence of the organelles was much weaker than in

the controls. The most potent fluorescence was found in the cytopyge(?), the least evident one in the cell membrane. In each sample white fluorescence was observed in both living and dead cells.

## Discussion

The findings pertaining to the effect of *Tetrahymena* preadaptation achieved in the presence of either colistin or ColDC upon the rate of food vacuole formation, indicate that the phenomenon known as "cross-adaptation" occurs in this case. Acquired resistance to a given type of toxin is the characteristic feature of phenotype adaptation. Thus, *Tetrahymena* cells adapted to cycloheximide, which inhibits protein biosynthesis, remain sensitive to colchicine, the antimiotic agent, and vice versa (Frankel 1970). However, they are resistant to antibiotics of different chemical structure, also acting on the ribosome, e.g., cycloheximide and emetine (Roberts and Orias 1974). Only in the case of inhibitors with a significant stereochemical affinity, such as, e.g. cycloheximide and streptomidone, action of one results in the adaptation to the presence of the other in the environment (Roberts and Orias 1974). Demonstrating the occurrence of "cross-adaptation" to colistin and ColDC in *Tetrahymena* is of a considerable importance since it allows to pretreat the cells with the colistin and then study the ciliate's response to ColDC.

Another question to be answered is: What is the mechanism (or mechanisms) which partly or completely inhibits incorporation of colistin into the cell membrane? Our results seem to indicate that the underlying process is saturation of the cell membrane with the antibiotic. The polymyxins, including colistin, bind with the lipopolysaccharide fragments of bacterial membrane (Kuryłowicz 1979). Lack of fluorescence of the *Tetrahymena* cell membrane in the presence of ColDC following a 24-h pretreatment with 16 mM colistin may suggest binding by colistin of all (specific to colistin in *Tetrahymena*) fragments of the membrane, thus precluding ColDC addition. Such a conclusion seems justified as colistin is likely to bind to the cell membrane in a stable manner. If the cells were incubated for 0.5 h or 1.5 h in the presence of 16 mM colistin, subsequently fixed in 2% $Os_2O_4$ and dialysed for 10 h in the presence of 0.9% NaCl, no fluorescence of the cell membrane was observed on addition 0.1 mM ColDC. On the other hand, a similar procedure performed with the cells which had received no pretreatment, produced fluorescence of the cell membrane and food vacuole membra-

ne, though much weaker than in living *Tetrahymena* (Szablewski — unpublished data).

It should be also explained why colistin effected blockade of the site of bind to ColDC only in the early log phase cells, whereas the phenomenon was not observed in the case of the same antibiotic concentrations acting upon the *Tetrahymena* derived from the other phases of growth. The fact might be associated with alteration in the chemical composition of lipids in the ageing *Tetrahymena* (Hill 1972). For instance, the fatty acids of *Tetrahymena* constitute about 5% of the dry weight of log-phase cells and about 10% of the dry weight of stationary-phase cells (Holz and Conner 1973). Possibly, in this case the increased antibiotic concentration during pretreatment would produce saturation of all chemical bonds specific for colistin and thus prevent ColDC incorporation into the cell membrane. However, because of colistin toxicity further increase in the antibiotic concentration during pretreatment is impossible. The conclusion is also confirmed by the comparison of fluorescence intensity observed in the control early log-phase cells and in the ciliates in the same growth phase, pretreated for 24 h in 8 mM colistin. In the latter fluorescence of the cell membrane was much weaker.

*Tetrahymena* reveal both quantitative and qualitative changes in lipids according to the growth phase (Holz and Conner 1973). For instance, the ratio of saturated to unsaturated fatty acids increase in the interval between early and late log phase (Erwin and Bloch 1963). These may be quantitative and qualitative lipid changes in *Tetrahymena*, which produce different colour of the cell membrane, varying with the growth phase.

A cultivation of *Tetrahymena* in the presence of some animal hormones causes the cell to be able to bind the hormones of higher animals by a receptor-like interaction, and it is usually also capable of a specific response to these hormones (Csaba et al. 1984). The evidence that the "receptor-like" membrane pattern of the *Tetrahymena* is a genuine receptor (Csaba et al. 1984) may suggest induction and subsequently saturation of these receptors under the influence of colistin. However, in the case of adaptation to colistin the mechanism is of a lesser importance, as indicated by the following observations: (1) the investigated substances (e.g., hormones) were acting at receptorial level in contrast to other substances which could be harmful for the cells (e.g., antibiotics); (2) the period of time necessary for the *Tetrahymena* recovery after the addition of colistin to the culture is shorter (Szablewski 1984, 1985) than the period of time required for the induction of "receptor-like" structures (Csaba et al. 1982 a); (3) The receptor "memory"

due to hormones persists through as many as 500 generations (Csaba et al. 1982 b). Adaptation of cells to e.g. cycloheximide makes the ciliates less sensitive to the repeated action of the antibiotic. However, the resistance decreases with each division and after a few generations *Tetrahymena* again becomes sensitive to cycloheximide (Roberts and Orias 1974).

Although in the case of *Tetrahymena* adaptation to colistin, mechanisms similar to those effected in the cell by hormones do not seem to play the most significant part, the role of the "receptor-like" membrane pattern due to the antibiotic action cannot be entirely excluded.

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# Factors Conditioning Biosynthesis of Prostaglandins in Pathogenic and Non-pathogenic Strains of *Acanthamoeba castellanii*

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*Synopsis.* The aim of the study was to investigate factors conditioning biosynthesis of respective prostaglandins in pathogenic (309) and non-pathogenic (Neff) strains of *Acanthamoeba castellanii*. Biochemical analysis proved that the rise of respective prostaglandins is conditioned by the enzymatic composition and factors present in cells. The course of biosynthesis *in vitro* can be activated by various compounds (glutathione, albumine, p-CMB) that are either activators or inhibitors of the enzymes. The course of biosynthesis *in vivo* is most probably activated by tissues or constitutional liquids surrounding the parasites.

Coordination of the function of cells of the same tissue or of structures within the cell is conditioned by external impulse or by interaction of the cells of the same or different species. These short-distance interactions are activated by the excretion of elements known as cell-hormones prostaglandins, among others) that strongly activate the metabolism of sensitive cells.

Prostaglandins, unsaturated fatty acids, play an important part in mammals and invertebrates (Horrobin 1978, Saintsings et al. 1981, Hokama et al. 1982) as well as in parasites (Miyares and Hollands 1976, Simonic et al. 1983, Grzywacz and Szkudliński 1986).

Studies conducted by many authors testify to the decisive role of the prostaglandins of parasites in metabolic processes. Those studies also

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showed that the prostaglandins of parasites can influence the invasion or penetration of the host (Leid and McConnell 1983 a, b, Salafsky et al. 1984).

The presence of prostaglandins in protozoa was established in 1977 (Das and Padma). Investigations conducted by the author of the present study established the composition of prostaglandins in pathogenic and non-pathogenic strains of *Acanthamoeba* spp. and presented the first stage of biosynthesis of these compounds (Hadaś 1987, 1988). It was established that pathogenic strains of *Acanthamoeba castellanii* produce greater quantities of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) than non-pathogenic strains though the oxidation of arachidonic acid — to the point prostaglandin H (PGH) being produced — is identical in both strains.

The aim of this study was to examine the factors that condition of several prostaglandins in pathogenic and non-pathogenic strains of *Acanthamoeba castellanii*.

## Material and Methods

Strains of amoebae: *Acanthamoeba castellanii*, strain 309, pathogenic for mice, isolated from cysts stored at 4°C from the time of the original isolation (Kasprzak and Mazur 1972), *Acanthamoeba castellanii*, strain Neff non-pathogenic for mice, sustained in axenic culture from the time of the original isolation (Neff 1957).

Amoebal culture: The amoebae were cultured axenically at 24°C in a liquid medium described by Cerva (1966), composed of 2% Bacto-Casitone (Difco) and 10% horse serum.

Homogenization: The amoebae harvested from 4 days cultures were centrifuged at 900 g for 5 min and subsequently washed with physiological saline and 100 mM Tris-HCl buffer of pH 8.2. The sediment of protozoa thus obtained was suspended in washing buffer at the ratio of 1 : 5 and homogenized in teflon homogenizer at 4°C. Thus homogenate was divided into two parts; one was immediately used in the investigation, and from the other a microsomal fraction was isolated.

Isolation of microsomal fraction: The previously obtained homogenate was centrifuged for 12 min at 12 000 g, then the sediment was disposed of and the supernatant fraction was centrifuged again for 60 min at 100 000 g. The supernatant fraction, free of microsomes, was stored separately, and the microsomal fraction was suspended to the initial volume of the homogenate with 100 mM of Tris-HCl buffer, pH 8.2.

Investigation of the biosynthesis of prostaglandins: Enzymatic incubation was conducted for 60 min at 37°C in mixture containing 0.8 ml of 100 mM Tris-HCl buffer with pH 8.2, 100 mM of arachidonic acid and 0.2 ml of microsomal preparation or homogenate. The enzymatic reaction was stopped by adding 0.1 ml 1 N HCl. As a control of the enzymatic reactions, preparations subjected to temperature of 100°C for 5 min were used.

Investigation of factors conditioning biosynthesis of prostaglandins: The following factors conditioning the course of biosynthesis of prostaglandins were investigated: (a) supernatant fraction, after microsomes were isolated; (b) the same supernatant fraction inactivated thermally; (c) 10% solution of bovine albumin; (d) 5 μM solution of p-chloromercuri benzoic acid (p-CMB); (e) 30 μM of glutathione solution. The investigated substances were solved in Tris-HCl incubating buffer.

Isolation and thin-layer chromatography of the products of enzymatic reactions: The products were extracted twice with 1.5 ml of ethyl acetate. After the organic phase had been vaporized, the residue was solved in 30 μl of methanol and deposited on glass plates covered with silica gel (Silica Gel 60 F — Merck). Chromatograms were developed by the following solvent system: ethyl acetate: acetic acid as 98 : 2, ethyl acetate: acetic acid: water as 16 : 1 : 10 or chloroform: methanol: acetic acid as 80 : 10 : 10. The chromatograms were made visible means of 3% cupric acetate in 15% water solution of phosphoric acid spray (Andersen 1969) or 10% alcoholic solution of phosphomolybdic acid spray and by heating to 120°C for 10 min. Visible stains of prostaglandins were identified according to patterns (Sigma) and according to the characteristic colours obtained with the cupric acetate spray (green for PGA and PGE, yellow for PGB, and violet for PGF).

Quantitative analysis of prostaglandins: It was carried out simultaneously with the qualitative examination. Extracts were separated on small columns packed with 0.5 g silicic acid (100-200 mesh, Sigma). The respective prostaglandins were eluated with 15 ml portion of solution in the following proportion: PGA and PGB — ethyl acetate: benzene as 3 : 7, PGE — ethyl acetate: benzene as 6 : 4, PGF — ethyl acetate: benzene as 8 : 2. The eluates were vaporized dry, solved in 50 μl of methanol and added to 1 ml of 1 N KOH. After 1 hour incubation the contents of prostaglandins were determined spectrophotometrically (Hamberg and Samuelsson 1966).

Isolation of prostaglandins from intact cells of amoebae: Amoebae obtained from 4-day cultures were rinsed with physiological saline and covered with 95% ethanol at the ratio of 1 : 10 and then homogenized. After 30 min, when proteins were precipitated, the alcoholic homogenate was centrifuged and the sediment was again treated with ethanol. Blended alcoholic extracts were vaporized almost dry and then suspended in a small quantity of distilled water. The extract was alcalized to pH 8.0 and extracted with the mixture of pentan and hexan at the ratio of 1 : 1 to get rid of fats and non-polar glycides. After the removal of fats, the water fraction was acidified to pH 3.5-4.0 and the prostaglandins were extracted twice with ethyl acetate and twice with ethyl ether. Blended organic extracts were vaporized dry, the residues were suspended in methanol to the original volume of amoebae and subjected to quantitative analysis in the above mentioned manner.

## Results

Thin-layer chromatographic method establisthed 5 clear spots that were identified as prostaglandin $E_2$ ($PGE_2$), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), prostaglandins $A_2$ and $B_2$ ($PGA_2$ + $PGB_2$), prostaglandin $D_2$ ($PGD_2$) and

metabolite of prostacycline ($PGI_2$), that is 6-keto-$PGF_{1\alpha}$. Table 1 presents $R_f$ values of prostaglandins for respective developing patterns.

Table 1

$R_f$ values of prostaglandins developed by thin-layer chromatography on silica gel (Silica Gel 60 F — Merck)

| Specifications | $R_f \times 100$ | | | | | |
|---|---|---|---|---|---|---|
| | $PGA_2$ | $PGB_2$ | $PGD_2$ | $PGE_2$ | $PGF_{2\alpha}$ | 6-keto $PGF_{1\alpha}$ |
| ethyl acetate:acetic acid 98:2 | 81 | 79 | 70 | 43 | 21 | 76 |
| ethyl acetate:methanol:water 16:1:10 | 82 | 80 | 74 | 51 | 30 | 79 |
| chloroform:methanol:acetic acid 80:10:10 | 90 | — | 75 | 73 | 56 | 70 |

Table 2 shows the influence of various activating agents on biosynthesis of prostaglandins (PGs). It was established that in homogenates and microsomes glutathione and p-CMB increase the biosynthesis of $PGF_{2\alpha}$ about two times in both examined strains. It was also established that glutathione does not substantially effect the production of $PGE_2$ and that p-CMB reduces the synthesis of $PGE_2$ by half. Bovine albumin is a week inhibitor of biosynthesis $PGF_{2\alpha}$ and minimally increases the production of $PGE_2$ and $PGA_2$ + $PGB_2$, the last two being determined globally in quantitative examination.

When the supernatant fraction, after microsomes were isolated, was added to incubation mixture, biosynthesis of respective PGs increases almost twice and the number of compounds is similar to that of homogenates. In the pathogenic strain (309) the increase of $PGF_{2\alpha}$ is by ca. 1.4 times whereas in the non-pathogenic strain (Neff) the increase of $PGE_2$ is by ca. 1.7 times.

Table 3 presents the results of quantitative analysis of prostaglandins isolated from intact amoebae.

The results of the quantitative examination of PGs formulated in the Tables are presented in μg/ml of agglomerated mass of amoebae to easier compare the homogenates with the microsomal fraction.

## Discussion

The basic product of the oxygenation of arachidonic acid by cyclooxygenase is endoperoxide — prostaglandin $H_2$ ($PGH_2$). It is a labile com-

Table 2

The influence of various activating agents on the biosynthesis of respective prostaglandins produced from 100 μM of arachidonic acid in homogenates and microsomal fractions. The contents of respective PGs are expressed in μg/ml of agglomerated mass of amoebae as mean value ± SD of seven experiments

| | Specification | $PGE_2$ | | | | $PGF_{2\alpha}$ | | | | $PGA_2+PGB_2$ | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | homogenate | | microsomal fraction | | homogenate | | microsomal fraction | | homogenate | | microsomal fraction | |
| | | value | SD | value | SD | value | SD | value | SD | value | SD | value | SD |
| *Acanthamoeba castellanii* strain 309 | Tris-HCl inactivated | 1.66 | 0.14 | 0.78 | 0.14 | 3.26 | 0.28 | 0.88 | 0.10 | 0.60 | 0.12 | 0.62 | 0.11 |
| | supernatant | | | 0.75 | 0.15 | | | 0.91 | 0.16 | | | 0.60 | 0.09 |
| | supernatant | | | 1.65 | 0.21 | | | 2.88 | 0.20 | | | 0.65 | 0.05 |
| | p-CMB | 0.90 | 0.09 | 0.84 | 0.08 | 3.54 | 0.22 | 1.21 | 0.21 | 0.63 | 0.08 | 0.61 | 0.06 |
| | glutathione | 1.89 | 0.10 | 0.85 | 0.14 | 3.80 | 0.22 | 1.40 | 0.13 | 0.96 | 0.10 | 0.63 | 0.05 |
| | albumin | 2.36 | 0.20 | 0.89 | 0.05 | 1.29 | 0.14 | 0.78 | 0.14 | 0.98 | 0.10 | 0.70 | 0.06 |
| strain Neff | Tris-HCl inactivated | 2.39 | 0.23 | 0.86 | 0.18 | 1.53 | 0.28 | 0.76 | 0.04 | 0.76 | 0.14 | 0.46 | 0.06 |
| | supernatant | | | 0.89 | 0.06 | | | 0.75 | 0.09 | | | 0.58 | 0.11 |
| | supernatant | | | 2.23 | 0.13 | | | 1.28 | 0.17 | | | 0.80 | 0.07 |
| | p-CMB | 0.91 | 0.11 | 0.78 | 0.07 | 2.51 | 0.42 | 1.03 | 0.10 | 0.80 | 0.14 | 0.63 | 0.05 |
| | glutathione | 2.53 | 0.32 | 0.86 | 0.16 | 3.31 | 0.32 | 1.29 | 0.14 | 1.03 | 0.17 | 0.65 | 0.15 |
| | albumin | 2.61 | 0.32 | 0.99 | 0.08 | 1.60 | 0.19 | 1.63 | 0.16 | 1.05 | 0.04 | 0.75 | 0.05 |

Table 3

Contents of respective prostaglandins isolated from intact cells of pathogenic (309) an non-pathogenic (Neff) strains of *Acanthamoeba castellanii* expressed in μg/ml agglomerated mass of amoebae as mean value ± SD of seven experiments

| Specifications | $PGE_1$ | $PGF_{2\alpha}$ | $PGA_2+PGB_2$ |
|---|---|---|---|
| strain 309 | 7.72 (±0.53) | 15.15 (±0.34) | 2.26 (±0.31) |
| strain Neff | 14.35 (±0.38) | 7.82 (±0.59) | 3.51 (±0.45) |

pound, easily transformed to indissoluble prostaglandins (Fig. 1). As a result of the oxygenation of arachidonic acid to $PGH_2$ equivalent quantities of the compound are produced both in the pathogenic and non-pathogenic strains of *Acanthamoeba castellanii* (H a d a ś 1988). However,

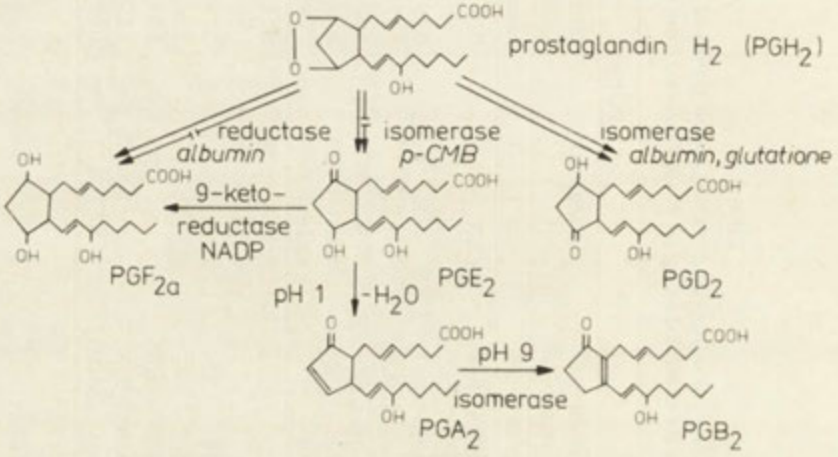


Fig. 1. Biosynthesis of respective prostaglandins from endoperoxide of prostaglandin H

earlier investigations (H a d a ś 1987) showed that bigger quantities of prostaglandin $F_{2\alpha}$ occur in pathogenic strains, whereas in non-pathogenic strains there occur bigger quantities of prostaglandin $E_2$.

The production of respective PGs in cells is possible either enzymatically or non-enzymatically. The present study was to state the degree to which the transformation of $PGH_2$ occurs in the cells of amoebae according to the above-mentioned procedures and to establish the influence of the examined compounds on the process.

It was established that the biosynthesis of respective PGs is to a large extent conditioned by factors present in the cytoplasma of cells. Adding inactivated supernatant after microsomes were isolated results in respective PGs being most probably created non-enzymatically. Transfor-

mation of $PGH_2$ to $PGF_{2\alpha}$ in cells is achieved through endoperoxide reductase, and the transformation of $PGH_2$ to $PGE_2$ and $PGD_2$ is achieved through endoperoxide isomerase (Decker 1985). Endoperoxide reductase can be activated with glutathione, whereas isomerases are sensitive to compounds catching such sulphydryl groups as, e.g., p-CMB. The transformation of prostaglandin H into solid PGs is also possible under the influence of glutathione S-transferase which is an enzyme connected with microsomes and whose presence was established in many worms (Douch and Buchanan 1978, Morello et al. 1982, Kawalek et al. 1984, Jaffe and Lambert 1986) as well as in protozoa (Yawetz and Agosin 1981).

Albumins that constitute the supply of cells may play certain role in the production of various prostaglandins. The transformation of $PGH_2$ to $PGF_{2\alpha}$ in mammals is retarded by the albumins of plasma and it is when $PGH_2$ isomerises to $PGD_2$ and $PGE_2$. The ratio of $PGD_2$ to $PGE_2$ increases with the growth of the concentration of albumin (Robak and Kasperczyk 1979). Kasprzak et al. (1986) demonstrated that a long-term culture of amoebae on artificial bedding with animal serum added deprives strains of *Acanthamoeba* spp. of their pathogenicity. It can be due to the fact that the synthesis of $PGF_{2\alpha}$ is inhibited by albumins and that the synthesis of $PGF_{2\alpha}$ can be responsible for pathogenicity. Similarly, ageing cells that store albumins lose their invasive propensity. The option is the production of $PGF_{2\alpha}$ from $PGE_2$ by 9-keto-reductase of PGs and the transformation $PGF_{2\alpha}$ to $PGE_2$ by dehydrogenation (Decker 1985).

Prostaglandin $D_2$ is a compound that comes into existence parallelly to $PGF_{2\alpha}$ and $PGE_2$. Small quantities of $PGD_2$ were demonstrated by thin-layer chromatography. However, because of a different method of its isolation on columns, $PGD_2$ was not determined quantitatively. In mammals it prevents the aggregation of blood platelets but nothing is known of its agency in protozoa.

The origins of $PGA_2$ and $PGB_2$, whose presence was demonstrated by this study, are connected with biosynthesis of $PGE_2$. Zaorska (1986) assumes that the transformation of $PGE_2$ to $PGA_2$ may occur non-enzymatically in acidic medium, e.g., during extraction. In alkaline medium $PGA_2$ is transformed to $PGB_2$. The role of $PGA_2$ and $PGB_2$ in mammals is little investigated. It is assumed that prostaglandin $A_2$ is a rotating hormone that plays osmoregulating role in achieving proper concentration of ions. It most probably plays similar role in amoebae (Das and Padma 1977). PGA and PGB are globally determined in the quantitative analysis. They constitute ca. 10-15% of the total contents of PGs.

In those enzymatic studies on isolated microsomes in which reduced glutathione was used as co-factor of the reaction, vestigial quantities of 6-keto-$PGF_{1\alpha}$ were found. The compound, however, was not found in intact amoebae. 6-keto-$PGF_{1\alpha}$ is in fact a metabolite of prostacycline ($PGI_2$) and its creation through the transformation of PGE, PGF and PGD seems to be of little likelihood. The origination of prostacycline in low concentration of $PGH_2$ was, however, demonstrated in isolated animal microsomes which were activated with glutathione (Cottee et al. 1977).

The composition of prostaglandins and their total contents in cells to a large extent depend upon the isolating methods used. The investigation proved that the number of prostaglandins in the intact cells treated with alcohol differs from that of the cells extracted after homogenization. The difference, however, was predictable as it is well known that each factor that damages a cell (mechanical, chemical or thermal) provokes a chain of reactions that lead to the exhaustion of PGs and to biosynthesis *de novo*. As a fatty acids, prostaglandins can also be used up as substrate to energic transformation and this was noticed as a decrease of the total contents of PG in homogenates.

Biochemical investigations of biosynthesis of respective prostaglandins in pathogenic (309) and non-pathogenic (Neff) strains of *Acanthamoeba castellanii* demonstrated that the rise of respective PG depends on the enzymatic composition of cells and that the extortion of the biosynthesis of an appropriate enzyme is present. It also seems that the course of biosynthesis in host can be differently activated depending on the tissue or constitutional liquid that surrounds the parasite.

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# Spatial Separation of Terrestrial Ciliates and Testaceans (*Protozoa*): a Contribution to Soil Ciliatostasis

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*Synopsis.* The vertical distribution of soil ciliates and testaceans was investigated in a spruce forest, in a meadow and at some alpine and lowland sites. A direct and an indirect (culture) method were used to count the organisms. Active ciliates are abundant in the litter (L) but low numbers occur in the F and H layer of the spruce forest. Testaceans, in contrast, reach peak numbers in the F and H horizon which causes a distinct spatial separation from the ciliates. No or very few active ciliates occur in the meadow and the other sites which have no litter layer. Cultures of air-dried and remoistened soil samples, however, nearly always yield high numbers. These findings can be explained with the concept of ciliatostasis and support the view that certain chemical substances which are present in older soils are mainly responsible for the inhibition of ciliate excystment and growth in most evolved natural soils. The annulment of ciliatostasis in cultures by drying and remoistening of the soil is perhaps achieved through the inactivation or disappearance (e.g., by evaporation) of these substances. The increased bacterial food supply may also be of some importance. However, under field conditions food is probably not the key factor, because food organisms (e.g., bacteria, fungi) are surely abundant in the upper layers of sites where no or only few active ciliates are found as in meadows and arable land. Likewise, pore space can be excluded.

With a direct counting method terrestrial ciliates and testaceans have virtually never been investigated together at the same site with the exception of works by Foissner (1981, 1985), Foissner and Adam (1981), Foissner et al. (1985, 1988) and his group (Berger et al. 1985,

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1986, Lüftenegger et al. 1986 a). Bamforth (e.g., 1971, 1984) studied both communities in cultures, but discrepancies between direct counts and culture methods make it difficult to draw conclusions on the field situation (Foissner 1987). Besides, hardly any results concerning an exact spatial distribution of ciliates and testaceans are known, because either very few active ciliates have been found in the soil or the investigated strata were too thick to get clear results. Therefore we studied the ciliates and testaceans of a spruce forest and a meadow in 0-1, 1-3 and 3-9 cm depth.

An additional reason for this investigation was to collect data on soil ciliatostasis. Ciliatostasis is a phenomenon of most evolved natural and cultivated soils and severely restricts excystment and growth of ciliates compared to their behavior under the same conditions of e.g., temperature, moisture, pH etc. in laboratory cultures (Foissner 1987). Currently, not much is known about the causes. Foissner (1987) suggested that unknown chemical substances, present mainly in evolved soils, could be responsible. The ecological implication may be to maintain an equilibrium between soil organisms as similar phenomena, termed soil microbiostasis, are already known for actinomycetes, fungi and bacteria (Ko and Chow 1976).

In his review Foissner (1987) founded the hypothesis in part on data from the literature and on unpublished observations, most of which are reported here.

## Materials and Methods

### Site Descriptions

Site 1: Spruce forest in Oberhaag near Aigen (Upper Austria), 860 m NN. 0-1 cm soil depth is the L layer and consists mainly of fresh needles (about 1 year old). 1-3 cm is a more compact layer of older and slightly decomposed needles (F layer). 3-9 cm is raw humus (H layer). Each zone was investigated at least 12 times for ciliates and 4 times for testaceans in Oct. and Nov. 1985 in the course of a precipitation experiment.

Sites 2-4: Meadows and arable land near Salzburg city, about 430 m NN. Detailed site descriptions in Foissner et al. (1988). At site 2 (meadow) 3 replicates of 1 sample date (Dec. 1985) were investigated. The 0-1 and 1-3 cm layers consist mainly of plant roots and plant residues, while 3-9 cm is mineral soil. At site 3 (meadow) and 4 (arable land) 8 samples were taken between Oct. 1983 and 1986. Type of humus: mull.

Sites 5-7: Grossglockner area, Hohe Tauern (Austrian Central Alps), investigated between June and Oct. 1978. Detailed site descriptions in Foissner (1981). At site 5, an alpine pasture in 1900 m NN, 5 samples were studied. At

sites 6 (alpine mat) and 7 (alpine pasture, heavily fertilized by waste water), about 2300 m NN, only 1 sample each was investigated. Type of humus: mull-like moder.

Sites 8-10: Tullnerfeld area, near Vienna, about 180 m NN. 10 samples were investigated between Aug. 1980 and Nov. 1982. Detailed site descriptions in Foissner et al. (1985). Site 8 is xerothermic (mull-like moder), site 9 is a bottomland (mull) inundated at least twice a year, and site 10 is arable land.

## Methods

Soil samples were taken and prepared according to Foissner (1985) and Foissner and Pear (1985). Direct counting method: 0.005-0.2 g fresh soil are suspended in a few ml of sterile soil solution and counted directly under a microscope at 40× (ciliates) and 100× magnification (testaceans). For detailed description of these methods see Lüftenegger et al. (1988). The culture method used is very similar to that described by Buitkamp (1979): Air-dried soil is saturated with water and ciliates are counted 6 days after culture set-up. However, we used different amounts of soil, therefore the absolute numbers obtained with this method (although per g dry mass) are probably not fully comparable. Nevertheless, these values are useful for a comparison with those of the direct counts. Within a site equal amounts of soil were used with both counting methods.

Soil moisture (% of wet mass of soil), organic matter (% of dry mass of soil) and pH were determined as described by Berger et al. (1986), loss-on-ignition (% of dry mass of soil) was ascertained at 550°C, bulk density was estimated as described by Foissner (1981).

## Results

Ciliates and testaceans are distinctly separated in the spruce forest (Table 1, Fig. 1). The highest numbers of active ciliates (max. 603 ind. $g^{-1}$ dry mass [dm], $\bar{x} = 350$) occur in the uppermost 0-1 cm. Numbers decrease ($p < 0.05$) with increasing soil depth. In 7 out of 15 samples from 3-9 cm active ciliates are absent. The testaceans, in contrast, reach highest individual densities between 1-3 cm (max. 41517 ind. $g^{-1}$ dm, $\bar{x} = 31408$) and lowest in the 0-1 cm layer. All samples contain active testaceans.

Only very few active ciliates occur in the meadows and the arable land (Table 2). However, with the culture method high numbers grow in all samples. Despite a detailed vertical investigation of site 2, no active ciliates were found in 0-1, 1-3 and 3-9 cm depth. The highest numbers of testaceans occur in the top 0-1 cm (3615 ind. $g^{-1}$ dm). With increasing soil depth numbers decrease sharply ($p < 0.05$). The same distribution is obtained for the ciliates if investigated with the culture method (Table 2).

Table 1

Arithmetic mean ± S.D. of the abundance of testaceans and ciliates and of environmental parameters in a spruce forest[a]

| Site | Soil depth (cm) | Testaceans[b] ($g^{-1}$ dm) | Ciliates direct count ($g^{-1}$ dm) | Soil moisture (%) | Loss-on-ignition (%) | pH | Bulk density (g $cm^{-3}$) |
|---|---|---|---|---|---|---|---|
| 1 Spruce forest | 0–1 | 11138*[c] (±1084) | 350* (±167) | 54.3 (±6.7) | 95.1 (±1.0) | 3.9 (±0.1) | 0.07 |
| | 1–3 | 31408* (±7427) | 109* (±116) | 54.3 (±8.9) | 89.8 (±0.6) | 3.9 (±0.0) | 0.05 |
| | 3–9 | 17385* (±4614) | 14* (±24.1) | 45.2 (±6.4) | 47.9 ±17.7) | 3.4 (±0.2) | 0.29 |

[a] — testaceans: n = 4; ciliates, soil moisture: n = 12–15; loss-on-ignition, pH: n = 3; bulk density: n = 1, [b] — only living individuals; dm = dry mass, [c] — different at $p < 0.05$ (*) with the H-test of Kruskal-Wallis (Köhler et al. 1984)

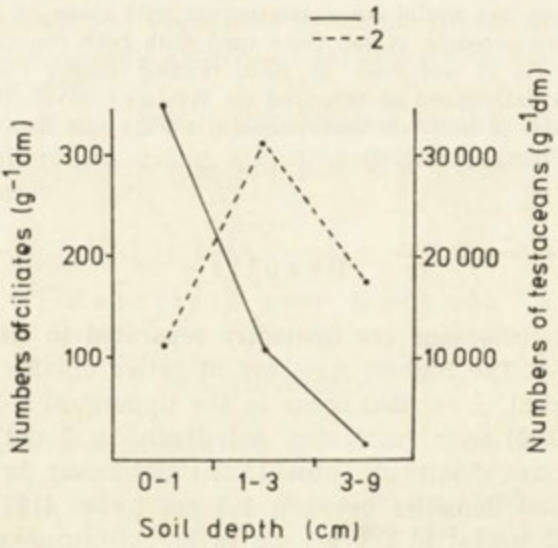


Fig. 1. Spatial separation of active ciliates (*1*) and testaceans (*2*) in a spruce forest

The striking difference between direct counting and the culture method is also demonstrated by an investigation of 3 alpine localities (sites 5-7, Table 3). No active ciliates occur in sites 6 and 7 but relatively high numbers are present in 0-2 cm of site 5 ($\bar{x}$ = 224 ind. $g^{-1}$ dm). The culture method provides excessively high values, especially for the 0-2 cm layers, at all 3 sites. Testaceans prefer the 2-4 cm depth both at sites 6 and 7.

Table 2

Arithmetic mean ± S.D. of the abundance of testaceans and ciliates and of environmental parameters in meadows and an arable land[a]

| Site | Soil depth (cm) | Testaceans[b] ($g^{-1}$ dm) | Ciliates ($g^{-1}$ dm) direct count | Ciliates ($g^{-1}$ dm) culture method | Soil moisture (%) | Organic matter (%) | pH | Bulk density ($g\ cm^{-3}$) |
|---|---|---|---|---|---|---|---|---|
| 2 Meadow | 0–1 | 3615*[c] (±1975) | 0 | 3112* (±697) | 41.0 (±1.4) | ND[d] | ND | ND |
| | 1–3 | 1436* (±329) | 0 | 1810* (±653) | 40.0 ±1.0) | ND | ND | ND |
| | 3–9 | 587* (±218) | 0 | 1241* (±912) | 37.8 (±0.3) | ND | ND | ND |
| 3 Meadow | 0–5 | 948 (±380) | 2 (±5.3) | 2235 (±3588) | 31.4 (±3.9) | 6.7 (±4.0) | 5.9 (±0.6) | 0.93 |
| 4 Arable land | 5–15 | 528 (±233) | 1 (±2.5) | 405 (±173) | 33.2 (±1.6) | 5.2 (±0.6) | 7.0 (±0.4) | 0.91 |

[a] – site 2: n = 3; sites 3, 4: n = 8; organic matter, pH: n = 5; bulk density: n = 1, [b] – only living individuals; dm = dry mass, [c] – different at $p < 0.05$ (*) with the H-test of Kruskal-Wallis (Köhler et al. 1984), d – not determined in each layer; in 0–5 cm: organic matter 7.0, pH 7.1, bulk density 0.78 g $cm^{-3}$

Generally, moderate to low numbers are found with the direct and the culture technique in the Tullnerfeld localities (sites 8-10, Table 3). Only site 8 yields considerable numbers in the cultures which are different at $p < 0.001$ from those of the direct counts. Similarly, these values differ in the 0-5 cm layer of site 10 ($p < 0.1$), whereas in the remaining strata of sites 9 and 10 no pronounced differences exist ($p > 0.1$).

## Discussion

The most surprising results are the sharp decrease in the abundance of the active ciliates between litter and slightly decomposed litter[2], and the nearly total lack of active ciliates in the uppermost humus horizon of the spruce forest (Table 1, Fig. 1) as well as in all strata of meadows and arable land (Tables 2, 3). On the contrary, testaceans favor the F and H layer of the forest (Table 1, Fig. 1) and the 2-4 cm horizons of sites 6 and 7 (Table 3). Lousier and Parkinson (1984) reported a very similar testacean distribution in a deciduous forest. Schönborn (1986) found a corresponding distribution of active ciliates in two coniferous forests. Brunberg-Nielsen (1968) reported up to 32550

[2] We observed this dramatic decline also in a single sample of a beech forest. In the 0-2 cm layer (leaves) 3326 active ciliates $g^{-1}$ dm were recorded and none between 2-4 cm (F/H layer); testaceans: 0-2 cm 17196 $g^{-1}$ dm, 2-4 cm 2645 $g^{-1}$ dm.

Table 3

Arithmetic mean±S.D. of the abundance of testaceans and ciliates and of environmental parameters in Grossglockner (5–7) and Tullnerfeld (8–10) localities[a]

| Site | Soil depth (cm) | Testaceans[b] ($g^{-1}$ dm) | Ciliates ($g^{-1}$ dm) | | Soil moisture (%) | Organic matter (%) | pH | Bulk density (g $cm^{-3}$) |
|---|---|---|---|---|---|---|---|---|
| | | | direct count | culture method | | | | |
| 5 Alpine pasture | 0–2 | 2457 (±1052) | 224 (±186) | 11654 (±9252) | 54.7 (±8.5) | in 0–10 cm | | 0.48 |
| | 2–10 | 435 (±313) | 6 (±8.2) | 1573 (±1211) | 33.6 (±5.3) | 5.5 | 5.8 | 0.81 |
| 6 Alpine mat | 0–2 | 3050 | 0 | 21700 | 53 | in 0–8 cm | | in 0–5 cm |
| | 2–4 | 4100 | 0 | 950 | 45 | 10.0 in 0–12 cm | | 0.59 |
| | 4–8 | 570 | 0 | 300 | 41 | | 4.7 | in 5–10 cm |
| | 8–12 | 30 | 0 | 0 | 27 | 4.9 | | 0.82 |
| 7 Eutrophic alpine pasture | 0–2 | 3400 | 0 | 14000 | 75 | ND[c] | ND | in 0–5 cm |
| | 2–4 | 3900 | 0 | 6500 | 62 | ND | ND | 0.25 |
| | 4–8 | 550 | 0 | 50 | 51 | ND | ND | in 5–10 cm |
| | 8–12 | 630 | 0 | 30 | 45 | ND | ND | 0.67 |
| 8 Xerothermic site[d] | 0–5 | 725 (±416) | 154 (±186) | 720** (±443) | 25.1 (±9.0) | 5.6 | 7.5 (±0.4) | 0.68 |
| | 5–10 | 167 (±123) | 44 (±72) | 603** (±674) | 19.0 (±3.9) | 3.6 | 7.6 (±0.4) | 0.99 |
| 9 Bottomland[d] | 0–5 | 1556 (±643) | 51 (±43) | 35 (±39) | 40.7 (±7.9) | 6.6 | 7.5 (±0.3) | 0.68 |
| | 5–10 | 859 (±342) | 16 (±20) | 34 (±34) | 33.5 (±2.5) | 6.9 | 7.6 (±0.3) | 0.81 |
| 10 Arable land[d] | 0–5 | 156 (±106) | 56 (±127) | 37* (±42) | 15.2 (±6.5) | 2.6 | 7.4 (±0.4) | ND |
| | 5–10 | 214 (±251) | 18 (± 28) | 23 (±30) | 17.8 (±8.3) | 2.8 | 7.6 (±0.4) | 1.16 |

[a] – site 5: active ciliates, testaceans n = 5; culture method, soil moisture n = 4; organic matter, pH, bulk density n = 1. Sites 6, 7: n = 1. Sites 8–10: n = 10; organic matter, bulk density n = 1, [b] – only living individuals; dm = dry mass, [c] – not determined, [d] – different at $p < 0.001$ (**) and $p < 0.1$ (*) from the direct count with the U-test of Mann-Whitney (Köhler et al. 1984), no difference at $p < 0.1$ in site 9 and in 5–10 cm of site 10 as well as in testacean abundance between 0–10 cm of site 10

active ciliates $g^{-1}$ dm in the L layer of a beech forest and somewhat less in the F horizon. These observations indicate a distinct spatial separation — at least in forests — between ciliates which prefer the L layer and testaceans which favor the F and H horizons.

How can this spatial separation be explained? *A priori*, one would expect a reverse vertical distribution because the testate amoebae have better adaptations than ciliates to resist desiccation which certainly occurs frequently in litter. At first glance, the quicker division and cystation capacities of the ciliates, which allow a more immediate response

to changed environmental conditions as compared to the testaceans, seem to account for the separation. However, how can one explain, then, that hardly any active ciliates are present in the humus layer of the spruce forest and in all strata of meadows and various other sites where a high testacean abundance indicates good living conditions; and last but not least, why do so many ciliates appear in cultures from these habitats (Tables 1-3)?

An appropriate explanation, at least partially, could be offered by ciliatostasis (see introduction). If one looks at the presented results and data from the literature, the findings reported here fit exactly into this concept: (1) High numbers of active ciliates in litters and a sharp decrease with increasing humification and therefore more evolved soil (Table 1, footnote 2). (2) Absence or low numbers of active ciliates in all strata of meadows and arable land (Tables 2, 3), where no litter layer exists, only evolved soil. Site 5 is an unusual exception if compared with other similar sites (e.g., Foissner 1985, Berger et al. 1985). (3) Nullification of ciliatostasis in the laboratory by drying and remoistening of the soil (Tables 2-4) and by addition of glucose to soil (Foissner

Table 4

Arithmetic mean ± S.D. of the abundance of ciliates in fresh and washed soil and in cultures

| Site | Soil depth (cm) | Ciliates ($g^{-1}$ dry mass) | | |
|---|---|---|---|---|
| | | fresh soil | washed soil | culture |
| Cushion plant | 0–5 | 11 | 30*a | 1570 |
| site n = 8 | | (±14.1) | (±31) | (±1910) |
| Alpine mat | 0–10 | 2 | 49** | 134 |
| n = 10 | | (±3.2) | (±42) | (±90) |

a – different at $0.1 < p < 0.2$ (*) and $p < 0.005$ (**) from the fresh soil and at $p < 0.01$ (**) from the culture with the U-test of Mann-Whitney (Köhler et al. 1984)

1987). These procedures enrich the substrate with energy-containing nutrients and cause an abundant growth of food organisms (e.g., bacteria, fungi) indicating that food could be an important factor in overcoming ciliatostasis. This is supported by fertilization experiments on a ski slope after top soil removal (Lüftenegger et al. 1986 a). However, the above mentioned points 1 and 2 argue against such an explanation because bacteria and fungi are surely abundant at sites where active ciliates are rarely encountered, like in the upper soil layers of meadows and in the humus layer of the forest. Besides, Foissner (1985) reported an adverse pattern of ciliate abundance and dehydrogenase activity and

no connection with catalase activity. That means that food, though certainly an important factor, is very probably not the main reason for the lack of active ciliates in certain soils. Lüftenegger et al. (1986 b) showed that top soil removal was necessary for the nullification of ciliatostasis in the ski slope. A certain portion of the high abundance obtained with the culture method results from multiplication of ciliates during the 6 days of incubation. However, it is known from investigations with Singh's (1946) dilution method that a high amount of cystic (inactive) protozoa exists in many soils.

Under field conditions, the crucial point seems to be the age of the soil, as Foissner (1987) already suggested. This exactly corresponds with the present observations that in non-evolved soil "litter" much higher numbers of active ciliates exist than in the more evolved F and H layers. These older horizons must contain a factor restricting excystment and growth of ciliates. A study by Foissner (1981) provides experimental indication of this. Small chambers with washed soil were buried at the original sites. After about 16 days they were recovered and ciliates were counted. The washed soil, although very probably containing less food, yielded more active ciliates than the fresh soil but numbers in cultures were still higher (Table 4). It is conceivable that a (chemical) factor responsible for ciliatostasis was diluted or partly washed out. Perhaps such a restricting substance is inactivated or lost (e.g., by evaporation) in laboratory cultures because rewetting of the soil hardly causes a dilution.

The influence of the age of the soil is stressed by data from site 9 and 10 (Table 3). Both the inundation of the bottomland and the tillage of the arable land produces a layer of "young" soil. Thus, as expected, the differences between direct and culture counts are comparatively low.

The age of the soil is probably not the only factor which is responsible for ciliatostasis. There are hints that ciliatostasis requires the presence of living microorganisms (Foissner 1987). But very likely, this and other parameters, e.g., soil moisture, organic matter, pH or soil density are of minor importance as shown by our data (Tables 1-3). Among these factors, especially soil density (or rather pore space) is believed to limit ciliate occurrence in the soil (Darbyshire 1976, Alabouvette et al. 1981). But field experiments by Berger et al. (1985) and our results (Tables 1-3) suggest that soil density is not crucial for the occurrence of active ciliates. Even in strata with very low bulk density, which proves plenty of larger pores (e.g., 3-9 cm of site 1 or 0-2 and 2-4 cm of site 7, Tables 1, 3), no or only very few active ciliates are encountered.

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# Morphological Variation in a Ciliate, *Trichodina reticulata* Hirschmann et Partsch, 1955 (*Peritrichida*), in Tadpoles from Small Ponds

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*Synopsis.* Analysis of variance made on 19 populations of *Trichodina reticulata* Hirschmann et Partsch from tadpoles has shown that seasonal variation affects most the cell dimensions. The ciliates from tadpoles were also compared with those from crucian carps described elsewhere (Kazubski 1982 a). Great differences in the adhesive disc and the denticulate ring diameters and lack of differences in the number of denticles were noted in populations from the beginning of summer while difference in the adhesive disc diameter and lack of differences in the denticulate ring diameter and the number of denticles were observed in populations from the end of summer. In all cases great interpopulational variation was noted. The source and mechanism of the observed variation among trichodinas are discussed.

Morphological variation of ciliates, especially of trichodinas, was the subject of earlier investigations summarized by Kazubski (1982 b and 1986). The present paper is a continuation of these investigations and concerns the problem of influence of the host species on ciliate morphology.

Trichodinas occurring on tadpoles are especially interesting objects of study on variation. These are species typically occurring on other hosts (e.g., hydras, fishes etc.). They form only temporary populations on tadpoles, lasting from spring till autumn. Due to this fact the variation observed in them may be attributed to the influence of new hosts and conditions in which they live.

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The present paper is devoted to variation in *Trichodina reticulata* Hirschmann et Partsch, 1955. This is a typical and common parasite of crucian carp, *Carassius carassius* (L.) but occurs also on other fish species and on amphibian tadpoles. There is no doubt that the populations of *T. reticulata* on tadpoles are formed by ciliates originating from crucian carps. Variation of *T. reticulata* on crucian carps has been investigated earlier (Kazubski 1982 a). Thus, there is opportunity to compare the present material with that studied earlier and to avaluate the influence of the host species on the morphology of the ciliate examined.

## Material and Methods

The trichodinas used in the present investigation were collected in 1963-1964 from tadpoles of various amphibians, mainly of *Rana temporaria* (L.) from a small pond in Kortowo near Olsztyn. From the same pond and time specimens of *Carassius carassius* also infected with *T. reticulata* were collected giving a source of the material described elsewhere (Kazubski 1982 a). This material was used for comparison with the present results.

The ciliates were collected from the end of May up to September. The precise dates are given in Table 1. The term "population" means here ciliates living on single host individual according to Kazubski (1982 b).

Body dimensions and dimensions of the adhesive disc were measured on silver stained preparations after Klein. As an optimum sample size 30 ciliates from a population were measured. Measurements were taken according to a previously adopted method (Kazubski 1979, 1982 b). The following characters were analyzed: (1) body diameter, (2) adhesive disc diameter with the border membrane, (3) adhesive disc diameter without the border membrane, (4) denticulate ring diameter, (5) number of denticles, and (6) denticle length. Additionally in each population the mean length of the arch of the denticulate ring corresponding to single denticle (width of denticle) was calculated according to the formula:

$$\frac{\text{denticulate ring diameter}}{\text{mean number of denticles}} \times \pi.$$

Variation was investigated using statistical methods, mainly the two level nested analysis of variance (according to Sokal and Rohlf 1981). Three characters were analyzed: (1) adhesive disc diameter without border membrane, (2) denticulate ring diameter, and (3) number of denticles. The choice of these three characters as representative for trichodinas was given by Kazubski (1979).

Variation was examined between groups of populations from the beginning of summer 1963 and 1964 and from the beginning and the end of summer in the whole material collected from tadpoles. Comparison was made also between trichodinas from tadpoles and those from crucian carps described earlier (Kazubski 1982 a). As seasonal variation was manifested by trichodinas from both host species the materials collected at the beginning and the end of summer were compared separately. In all cases the interpopulational variation of trichodinas was also examined. In order to test a hypothesis about the occurrence of differences between discerned groups of trichodinas the analysis of variance was done.

## Results

The values of metric and meristic characters of 19 populations of *T. reticulata* from tadpoles are given in Table 1. This Table contains mean values and standard deviations of particular populations examined as well as of groups of populations. The same data concerning the whole material are also comprised.

Most populations of trichodina examined were collected from *R. temporaria* tadpoles, only single populations originated from *R. esculenta* s.l., *Bufo bufo* (L.) and *Triturus vulgaris* (L.). These populations did not differ in cell dimensions and number of denticles from those from *R. temporaria* and thus were treated together in further consideration.

Even a rough analysis of the data given in Table 1 showed a great diversity of mean values for particular populations. In the whole material these differences ranged from 20.5 to 26.3% of the smallest value. Differences in mean values of particular characters between groups of populations were also readily apparent. The differences between groups of populations collected in early summer of two succeeding years 1963 and 1964 (populations No. 12-16 and 40-56) were not great, varying from 0.47% in denticle length, 1.88% in body diameter, to 4.04% in the number of denticles in relation to the smaller value. These differences were greater when the populations from the beginning of summer (both years treated together) and the end of summer (populations No. 57-64 and 20) were compared, amounting to 6.91% in denticle length, 8.02% in the number of denticles and 17.76% in the denticulate ring diameter. In all cases analysed the mean values counted for populations from the end of summer (August-September) were greater than the corresponding data from the beginning of summer. Also the width of denticles counted according to the formula (1) was greater in ciliates in late summer.

These observations were ascertained by statistical analysis of variance. The analysis based on 10 populations from the beginning of summer (Table 2) has shown a lack of statistically significant differences between all values of characters in groups of populations from corresponding seasons of 1963 and 1964. It has shown also lack of significant differences in the adhesive dics diameter and the denticulate disc diameter between populations, while fairly great differences in the number of denticles exist. In the last mentioned character the variance ratio exceeded the critical value at 1% risk of error more than 4 times.

The analysis of variance for the whole material of 19 populations (Table 3) has shown significant differences between groups of populations from the beginning and the end of summer as well as between particular populations. The greatest differences between groups of po-

Table 1

Mean values (M) and standard deviation (SD) of main characters of examined populations of *Trichodina reticulata* from tadpoles from small pond in Kortowo (n = sample numerosity)

| Host species | Date | No of tadpoles | Diameter (μm) | | | | | | | | | No of denticles | | | Length of denticles | | | Width of denticles (μm) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | body | | | adhesive disc with border membrane | | | adhesive disc | | | denticulate ring | | | | | | |
| | | | M | SD | n | M | SD | n | M | SD | n | M | SD | n | M | SD | n | M | SD | n | |
| *Rana temporaria* | 26.6.63 | 12 | 68.25 | 9.47 | 24 | 55.12 | 5.30 | 25 | 48.10 | 5.16 | 29 | 30.02 | 3.52 | 30 | 22.33 | 1.42 | 30 | 15.19 | 1.02 | 26 | 4.22 |
| ,, | ,, | 13 | 70.89 | 8.09 | 28 | 54.36 | 4.59 | 28 | 47.83 | 3.87 | 30 | 30.76 | 2.68 | 31 | 24.00 | 1.39 | 30 | 14.00 | 0.72 | 30 | 4.03 |
| ,, | ,, | 14 | 74.51 | 8.58 | 29 | 55.21 | 4.23 | 29 | 47.14 | 3.98 | 29 | 30.55 | 2.89 | 29 | 23.93 | 1.46 | 30 | 14.97 | 0.99 | 29 | 4.01 |
| ,, | ,, | 15 | 73.07 | 9.06 | 27 | 56.79 | 5.58 | 29 | 48.60 | 5.64 | 30 | 31.02 | 4.02 | 30 | 24.58 | 1.20 | 30 | 15.11 | 0.92 | 28 | 3.96 |
| ,, | ,, | 16 | 71.07 | 5.98 | 30 | 54.67 | 4.05 | 30 | 46.23 | 3.59 | 30 | 29.67 | 2.73 | 30 | 24.00 | 0.87 | 30 | 14.73 | 0.63 | 30 | 3.88 |
| total, June 1963 | | | 71.66 | 8.33 | 138 | 55.23 | 7.65 | 141 | 47.58 | 4.53 | 148 | 30.40 | 3.21 | 150 | 23.79 | 1.48 | 151 | 14.93 | 0.87 | 143 | 4.01 |
| *Rana temporaria* | 30.5.64 | 40 | 69.74 | 7.20 | 19 | 56.37 | 3.79 | 19 | 48.55 | 3.26 | 22 | 30.93 | 2.15 | 22 | 24.45 | 1.18 | 22 | 14.91 | 0.81 | 22 | 3.97 |
| ,, | ,, | 41 | 72.35 | 8.40 | 20 | 56.00 | 4.38 | 19 | 47.62 | 3.10 | 25 | 29.96 | 1.98 | 27 | 23.93 | 1.17 | 27 | 15.02 | 0.93 | 22 | 3.93 |
| *Bufo bufo* | 17.6.64 | 50 | 73.43 | 5.83 | 21 | 56.75 | 3.01 | 20 | 49.54 | 3.18 | 24 | 31.19 | 1.71 | 24 | 24.79 | 1.06 | 24 | 14.47 | 0.77 | 19 | 3.95 |
| *Rana temporaria* | 11.7.64 | 55 | 76.39 | 8.26 | 18 | 57.71 | 5.96 | 17 | 49.19 | 4.77 | 21 | 31.35 | 3.23 | 26 | 25.13 | 1.19 | 24 | 14.79 | 1.12 | 14 | 3.92 |
| *Triturus vulgaris* | ,, | 56 | 73.33 | 9.93 | 18 | 57.00 | 4.46 | 16 | 49.94 | 4.42 | 17 | 32.18 | 3.38 | 17 | 25.82 | 1.01 | 17 | 15.60 | 1.52 | 5 | 3.92 |
| total 30.5–11.7.64 | | | 73.01 | 8.09 | 96 | 56.74 | 4.31 | 91 | 48.89 | 3.76 | 109 | 31.03 | 2.57 | 116 | 24.75 | 1.27 | 114 | 14.86 | 0.96 | 82 | 3.94 |
| total, June–July 1963–1964 | | | 72.22 | 8.24 | 234 | 55.82 | 6.58 | 232 | 48.14 | 4.26 | 257 | 30.68 | 2.96 | 266 | 24.20 | 1.47 | 265 | 14.91 | 0.90 | 225 | 3.98 |

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Rana temporaria* | 24.8.64 | 57 | 77.21 | 8.81 | 24 | 60.26 | 4.34 | 23 | 52.79 | 4.38 | 22 | 33.26 | 3.43 | 31 | 23.84 | 2.10 | 31 | 15.77 | 1.07 | 22 | 4.38 |
| „ | „ | 58 | 77.33 | 7.93 | 18 | 62.14 | 4.45 | 21 | 54.70 | 4.48 | 27 | 35.97 | 3.77 | 30 | 26.10 | 1.58 | 30 | 15.22 | 0.85 | 27 | 4.33 |
| „ | „ | 59 | 84.00 | 7.05 | 13 | 64.56 | 4.66 | 18 | 57.00 | 4.57 | 19 | 37.02 | 3.01 | 21 | 26.86 | 1.56 | 21 | 15.41 | 1.12 | 17 | 4.33 |
| „ | 25.8.64 | 60 | 82.25 | 10.26 | 12 | 62.31 | 5.44 | 16 | 54.38 | 5.07 | 21 | 35.31 | 4.32 | 26 | 26.19 | 1.74 | 26 | 15.28 | 0.98 | 25 | 4.24 |
| „ | „ | 61 | 82.07 | 4.55 | 14 | 65.58 | 3.96 | 26 | 58.41 | 3.88 | 29 | 40.03 | 3.83 | 30 | 27.60 | 2.06 | 30 | 16.87 | 1.20 | 30 | 4.56 |
| „ | „ | 62 | 84.96 | 9.42 | 25 | 61.31 | 4.46 | 29 | 54.53 | 4.25 | 30 | 35.17 | 3.02 | 30 | 26.00 | 2.18 | 30 | 16.53 | 1.01 | 30 | 4.25 |
| „ | „ | 63 | 75.39 | 9.13 | 28 | 60.83 | 5.10 | 30 | 54.83 | 4.74 | 30 | 35.90 | 2.82 | 30 | 27.30 | 1.02 | 30 | 15.87 | 1.01 | 30 | 4.13 |
| „ | „ | 64 | 80.63 | 9.64 | 19 | 64.08 | 5.49 | 24 | 56.60 | 4.95 | 30 | 37.05 | 2.80 | 30 | 26.60 | 2.27 | 30 | 16.00 | 0.98 | 30 | 4.38 |
| *Rana esculenta* | 21.9.63 | 20 | 79.43 | 11.74 | 7 | 62.90 | 4.63 | 10 | 55.97 | 5.76 | 38 | 35.86 | 4.18 | 49 | 25.43 | 2.35 | 46 | 16.01 | 1.23 | 44 | 4.43 |
| total, August–September | | | 79.98 | 9.25 | 160 | 62.56 | 4.97 | 197 | 55.48 | 4.93 | 252 | 36.13 | 3.99 | 274 | 26.14 | 2.21 | 274 | 15.94 | 1.17 | 255 | 4.34 |
| total | | | 75.37 | 9.46 | 394 | 58.92 | 6.78 | 429 | 51.77 | 5.89 | 509 | 33.44 | 4.45 | 540 | 25.19 | 2.12 | 539 | 15.46 | 1.17 | 480 | 4.17 |

Percent differences between minimum–maximum mean values

| | | | | | | |
|---|---|---|---|---|---|---|
| in the whole material | 20.51 | 20.65 | 26.34 | 24.87 | 23.60 | 20.50 | |
| at the beginning of summer | 1.88 | 2.73 | 2.75 | 2.07 | 4.04 | 0.47 | |
| between beginning and end of summer | 10.74 | 12.07 | 15.25 | 17.76 | 8.02 | 6.91 | |

Table 2

Two-level nested ANOVA table for three examined characters of two groups of populations of *Trichodina reticulata* from tadpoles from the beginning of summer

| Source of variation | Degree of freedom | $F_s$ value | | | Critical value | |
|---|---|---|---|---|---|---|
| | | diameter of adhesive dis | diameter of denticulate ring | number of denticles | $F_{0.01}$ | $F_{0.05}$ |
| Among groups of populations | 2−1 = 1 | 4.951 ns | 2.240 ns | 3.586 ns | 11.259 | 5.320 |
| Among particular populations | 10−2 = 8 | 1.236 ns | 1.357 ns | 11.148 s | 2.62 | 1.985 |
| Within populations | n−10 | — | — | — | | |
| | | n = 257 | n = 266 | n = 265 | | |

n — sample numerosity

Table 3

Two-level nested ANOVA table for three examined characters of two groups of populations of *Thrichodina reticulata* from tadpoles from the beginning and the end of summer

| Source of variation | Degree of freedom | $F_s$ or F's value | | | Critical value | |
|---|---|---|---|---|---|---|
| | | diameter of adhesive disc | diameter of denticulate ring | number of denticles | $F_{0.01}$ | $F_{0.05}$ |
| Among groups of populations* | 2−1 = 1 | 125.243 s | 71.313 s | 16.517 s | 8.400 | 4.451 |
| Among partilar populations** | 19−2 = 17 | 2.660 s | 4.945 s | 10.964 s | 2.070 | 1.664 |
| Within populations | n−19 | — | — | — | | |
| | | n = 509 | n = 540 | n = 539 | | |

n — sample numerosity.

Due to unequal size of samples variance ratio for groups * with Satterthwaite's approximation is calculated; the degrees of freedom for populations ** calculated according to the same formula for each characters are 16.52, 16.73 and 16.87 respectively.

pulations were noted in the adhesive disc diameter (variance ratio exceeded $F_{0.01}$ value 14.91 times), great differences were noted in denticulate ring diameter (variance ratio exceeded the $F_{0.01}$ value 8.49 times) and fairly small in the number of denticles (variance ratio exceeded the $F_{0.01}$ value only 1.97 times). The opposite relations were obtained when the variation between particular populations was analyzed — the difference in the adhesive disc diameter was the smallest (variance ratio exceeded the $F_{0.01}$ value 1.29 times), difference in the denticulate ring diameter was slightly greater (variance ratio exceeded the $F_{0.01}$ value 2.39 times) and the greatest in the number of denticles (variance ratio exceeded the $F_{0.01}$ value 5.3 times).

The methods of description of the trichodina populations and statistic methods used for analysis of their variation allows comparison of the material from the present investigation with those described in other publications and to reveal some new factors responsible for variation in these ciliates. In the present investigation *T. reticulata* from tadpoles were compared with the same sepcies occuring on crucian carps, described earlier (K a z u b s k i 1982 a), collected from the same pond and in the same time. The corresponding data are given in Table 1 of the cited paper (K a z u b s k i 1982 a, p. 3). In both cases some seasonal changes in values of particular characters have been observed. The comparison is based on the corresponding seasonal groups in the following way: in the beginning of summer the trichodinas from tadpoles collected from the end of May to mid July are compared with trichodinas from crucian carp collected in June, July and August, in the late summer the trichodinas from tadpoles collected in August-September are compared with those from crucian carps collected in September. Mean values of all characters of groups compared and their percentage relations are given in Table 4.

At the beginning of summer the trichodinas from tadpoles had smaller body dimensions, adhesive disc and denticulate ring diameters than the trichodinas from crucian carp. At the end of summer the differences showed the same tendency but were less pronounced. In both cases the difference in the number of denticles was small. At the beginning of summer the trichodinas from tadpoles had smaller number of denticles than those from crucian carps while the opposite situation was observed at the end of summer.

The analysis of variance of three characters (adhesive disc diameter, denticulate ring diameter and number of denticles) gave the following results. In the beginning of summer (Table 5) the adhesive disc diameter of trichodinas from tadpoles and crucian carps differed greatly and the variance ratio exceeded the $F_{0.01}$ value over 65 times. Similarly in the

Table 4

Mean values of six examined characters of *Trichodina reticulata* from crucian carp and tadpoles at the beginning and the end of summer, and relation between these values

| Character | Beginning of summer | | | End of summer | | |
|---|---|---|---|---|---|---|
| | crucian carp (c) | tadpoles (t) | t/c ratio in % | crucian carp | tadpoles | t/c ratio in % |
| Diameter of the body | 81.79 | 72.22 | 88.30 | 86.36 | 79.98 | 92.61 |
| Diameter of the adhesive disc with border membrane | 65.52 | 55.82 | 85.20 | 68.25 | 62.56 | 91.66 |
| Diameter of the adhesive disc | 60.48 | 48.14 | 79.60 | 63.28 | 55.48 | 87.67 |
| Diameter of the denticulate ring | 34.71 | 30.68 | 88.39 | 36.55 | 36.13 | 98.85 |
| Number of denticles | 25.38 | 24.20 | 95.35 | 25.11 | 26.14 | 104.10 |
| Length of denticles | 17.80 | 14.91 | 83.76 | 18.07 | 15.94 | 88.21 |

Table 5

Two-level nested ANOVA table for three examined characters of two groups of populations of *Trichodina reticulata* from tadpoles and crucian carp from the beginning of summer

| Source of variation | Degree of freedom | $F_s$ or F's value | | | Critical value | |
|---|---|---|---|---|---|---|
| | | diameter of adhesive disc | diameter of denticulate ring | number of denticles | $F_{0.01}$ | $F_{0.05}$ |
| Among groups of populations* | 2−1=1 | 533.880 s | 79.074 s | 4.126 ns | 8.185 | 4.381 |
| Among particular populations** | 21−2=19 | 1.875 s | 2.834 s | 35.906 s | 2.006 | 1.630 |
| Within populations | n−21 | — | — | — | | |
| | | n = 571 | n = 580 | n = 579 | | |

n — sample numerosity.

Due to unequal size of samples variance ratio for groups * with Satterthwaite's approximation is calculated; the degrees of freedom for populations ** calculated according to the same formula for each characters are 18.70, 18.77 and 18.98 respectively.

case of denticulate ring diameter the variance ratio exceeded $F_{0.01}$ value about 10 times, while the difference in the number of denticles was not significant even at 5% risk of error. At the end of summer (Table 6) only the adhesive disc diameter in trichodinas from tadpoles and crucian carps differed significantly, and the variance ratio exceeded the

Table 6

Two-level nested ANOVA table for three examined characters of two groups of populations of *Trichodina reticulata* from tadpoles and crucian carp from the end of summer

| Source of variation | Degree of freedom | $F_s$ or F's value | | | Critical value | |
|---|---|---|---|---|---|---|
| | | diameter of adhesive disc | diameter of denticulate ring | number of denticles | $F_{0.01}$ | $F_{0.05}$ |
| Among groups of populations* | 2−1=1 | 37.194 s | 0.141 ns | 2.263 ns | 9.646 | 4.884 |
| Among particular populations** | 13−2=11 | 4.266 s | 5.889 s | 8.534 s | 2.280 | 1.830 |
| Within populations | n−13 | — | — | — | | |
| | | n = 336 | n = 358 | n = 358 | | |

n − sample numerosity.

Due to unequal size of samples variation ratio for groups * with Satterthwaite's approximation is calculated; the degree of freedom for populations ** calculated according to the same formula for diameter of adhesive disc is 10.95.

$F_{0.01}$ value about 3.5 times. Differences in denticulate ring diameter and number of denticles between both host groups were statistically insignificant. In all cases the differences between particular populations were significant and the variance ratio usually exceeded the critical value at 1% risk of error. It ought to be mentioned that greatest differences concerned the number of denticles.

## Discussion

*Trichodina reticulata* is a typical, widespread parasite of crucian carp occurring on the surface of fishes. It may parasitize also other fish species (Stein 1984) and amphibian tadpoles. On the latter it forms temporary populations lasting only to the metamorphosis of the hosts in summer (*Rana temporaria*, *R. arvalis*) or early autumn (*R. esculenta*, *Bufo* sp. sp.). These populations originate from ciliates occurring on fishes, mainly on crucian carps. They form a good model for investigation on variation. The changes appear always anew, as a direct result of colonisation of the new hosts. It is not possible to consider them as permanently adapted to various hosts or to suppose that the differences observed are a result of long-lasting selection. Of course, conditions of the outer environment, especially temperature, ought to be taken into account as always in the case of poikilothermic organisms.

First of all, no differences have been found between populations of *T. reticulata* living on various species of tadpoles. This shows that the

conditions found on these hosts are identical or very similar. Simultaneously, great differences between particular populations have been observed amounting to 26% of the minimum value for each character. Similar differences, ranging from 13 to 26% were counted for *T. reticulata* populations from crucian carp (Table 7, counted on the data from Table 1, Kazubski 1982 a, p. 3).

Table 7

Percent differences between minimum — maximum mean values of six examined characters of *Trichodina reticulata* from crucian carp (according to Table 1, Kazubski 1982)

| Diameter (μm) | | | | No. of denticles | Length of denticles |
|---|---|---|---|---|---|
| body | adhesive disc with border membrane | adhesive disc | denticulate ring | | |
| 21.20 | 17.07 | 18.00 | 26.12 | 18.59 | 13.30 |

In *T. reticulata* from tadpoles no statistically significant differences were noted between groups from two succeeding years (1963 and 1964). Instead, well marked differences were observd between means counted for the material collected in the beginning and the end of summer. It indicates that these ciliates are subjected to seasonal variation manifesting in the increase of cell dimensions and dimensions of its elements and the increase of the number of denticles towards the end of summer. Such a phenomenon was observed also in other trichodinas (Kazubski 1982 b).

Similar variation was noted in *T. reticulata* from crucian carp (Kazubski 1982 a). However, it ought to be mentioned that the clear increase of values of characters examined in *T. reticulata* from tadpoles took place earlier (at the turn of July and August) than in the case of *T. reticulata* from crucian carp (August-September). It is worth noting that the trichodinas from tadpoles show the greatest seasonal differences in adhesive disc diameter and denticulate ring diameter and less so in the number of denticles. The last mentioned feature is simultaneously characterized by greatest interpopulational variation. It proves that outer factors have a bearing only on cell dimensions of trichodinas while the number of denticles is managed by genetic factors rather. It proves also that between particular populations of trichodinas living on tadpoles fairly great isolation occurs.

Comparison of *T. reticulata* from tadpoles (present investigation) with the data from crucian carp (Kazubski 1982 a) revealed great influence of the host species on the cell dimensions of particular elements of the ciliate body but not on the number of denticles. The differences

in the cell dimensions were especially great at the beginning of summer while at the end of summer they were less pronounced being reduced only to differences in the adhesive disc diameter. Such a situation seems to suggest that the main cause of these changes are some factors of the outer environment bearing on ciliates in various circumstances, rather than another host species, differences in host physiology etc. It is possible that the habitat in which tadpoles usually stay at the beginning of summer (well heated water in shallow parts of ponds) causes quick growth of ciliate populations connected with the decrease of cell dimensions. With this in mind, the fairly great stability of the number of denticles is interesting, as well as variation of this feature in particular populations. This may be interpreted by genetic factors, effect of the founder or genetic drift and prove that the populations investigated show a similarity to isolated populations described in other species of trichodinas (K a z u b s k i 1982 b).

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# Morphologie und Infraciliatur von *Dileptus orientalis* sp. n., einem Bodenciliaten aus Qingdao, China

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*Synopsis.* Die Morphologie und Infraciliatur von *Dileptus orientalis* sp. n. aus dem Edaphon eines Standortes in Qingdao, China, wird beschrieben. *Dileptus orientalis* sp. n. ist durch folgende Merkmale charakterisiert: die Infraciliatur besteht aus 15-19 Somakineten mit einer dreireihigen Dorsalbürste, die Toxicysten sind artcharakteristisch sehr kurz und spindelförmig. Die Anzahl der kontraktilen Vakuolen schwankt zwischen 5 und 8, sie erstrecken sich bis die Rüsselspitze. Das Hinterende ist breit gerundet oder schwach zugespitzt. Die Größe beträgt 150-250 μm. Der Makronucleus besteht aus zwei Teilen, mit einem Mikronucleus dazwischen.

Morphologisch-taxonomische Untersuchungen ander Gattung *Dileptus* wurden u.a. durchgeführt von Kahl (1935), Dragesco (1963, 1986), Golińska (1966, 1971), Jankowski (1967), Kink (1973), Foissner (1981, 1984) und Wirnsberger et al. (1984).

Zur Artabgrenzung wurden herangezogen: Habitat, Größe, Anzahl und Form der Makronuclei. Diese Merkmale sind nach Foissner (1984) und unseren eigenen Beobachtungen aber sehr variabel.

Da bisher nur wenige Species der artenreichen Gattung *Dileptus* (ca. 40 Arten) mit Hilfe moderner Methoden untersucht worden sind (Dragesco 1963, Golińska 1971, Foissner 1984, Wirnsberger et al. 1984), soll in dieser Arbeit *Dileptus orientalis* durch Darstellung der Infraciliatur, Form der Extrusome (Toxicysten), biometrische Analyse und Lebendbeobachtung von anderen *Dileptus*-Arten abgegrenzt werden.

## Material und Methode

Die hier beschriebene Art stammt aus Böden der V. R. China, und zwar aus dem nördlichen Hang eines Hügels in der Stadt Qingdao. Das Bodenmaterial wurde am 11.09.86 in 0-3 cm Tiefe genommen, luftgetrocknet und in Plastikbeuteln nach Bonn transportiert.

Zur Anreicherung und Kultivierung der Bodenciliaten wurde das Probenmaterial in Petrischalen mit Leitungswasser im Verhältnis 1:5 aufgeschwemmt. Dem Versuchsansatz wurde ein Reiskorn beigegeben, um so die Versorgung mit Bakterien für Bakterienfresser zu gewährleisten.

Zur Untersuchung der Infraciliatur wurden Präparate nach der Protargolmethode (Wilbert 1975) und der trockene Silberimprägnation nach Foissner (1976) angefertig sowie eine eingehende Lebendbeobachtung vorgenommen.

Die Beschreibung der Art bedient sich der Terminologie von Corliss (1979), Curds (1982), Dragesco (1986) und Foissner (1984).

Abkürzungen der biometrischen Analyse:

Extrem.: Extremwert
$\bar{x}$: arithmetisches Mittel
M: Median
Sx: Standardabweichung
$S\bar{x}$: Standardfehler des arithmet. Mittels
V: Variabilitätskoeffizient
n: Anzahl der untersuchten Individuen

## *Dileptus orientalis* sp. n.

### Morphologie (Abb. 1a-h)

Größe *in vivo* 150-250 µm. Der Körper ist langestreckt, der Rumpf sehr flexibel (Tab. 1). Cilienlänge 8-10 µm. Der Rüssel ist etwa 2/5 körperlang. Das Schwanzende ist meist rundlich, seltener schwach zugespitt, dabei wenig bis deutlich abgeflacht. Das Tier bewegt sich träge. Das Plasma ist farblos bis gelblich, dicht unter der Pellicula sind viele winzige Granula unregelmäßig angeordnet. Der Kernapparat liegt zentral, bei der untersuchten Population immer aus zwei stabförmigen, oft leicht gebogenen Makronucleus-Teilen bestehend. Zwischen den beiden Makronucleus-Teilen liegt der kugelförmige Mikronucleus. Die Anzahl der kontraktilen Vakuolen schwankt zwischen 5-8, sie sind bis in den Rüssel verbreitet. Dabei sind die im Hinterende liegenden kontraktilen Vakuolen manchmal größer als die im Vorderende. Ein arttypisches Merkmal sind die extrem kurzen (1-2 µm), spindelförmigen Toxicysten, die in mehreren Reihen circumoral angeordnet sind. Ausgeschleudert sind sie stabförmig (Abb. 1 c). Das Entoplasma zeigt häufig mehrere Nahrungsvakuolen gelblicher Färbung. Im Hinterende ist manchmal eine große „Nahrungsvakuole" zu sehen, die auf den ersten Blick einer kontraktilen Vakuole ähnelt. Es handelt sich hier vermutlich um die Cytopyge. Das Tier frißt Bakterien.

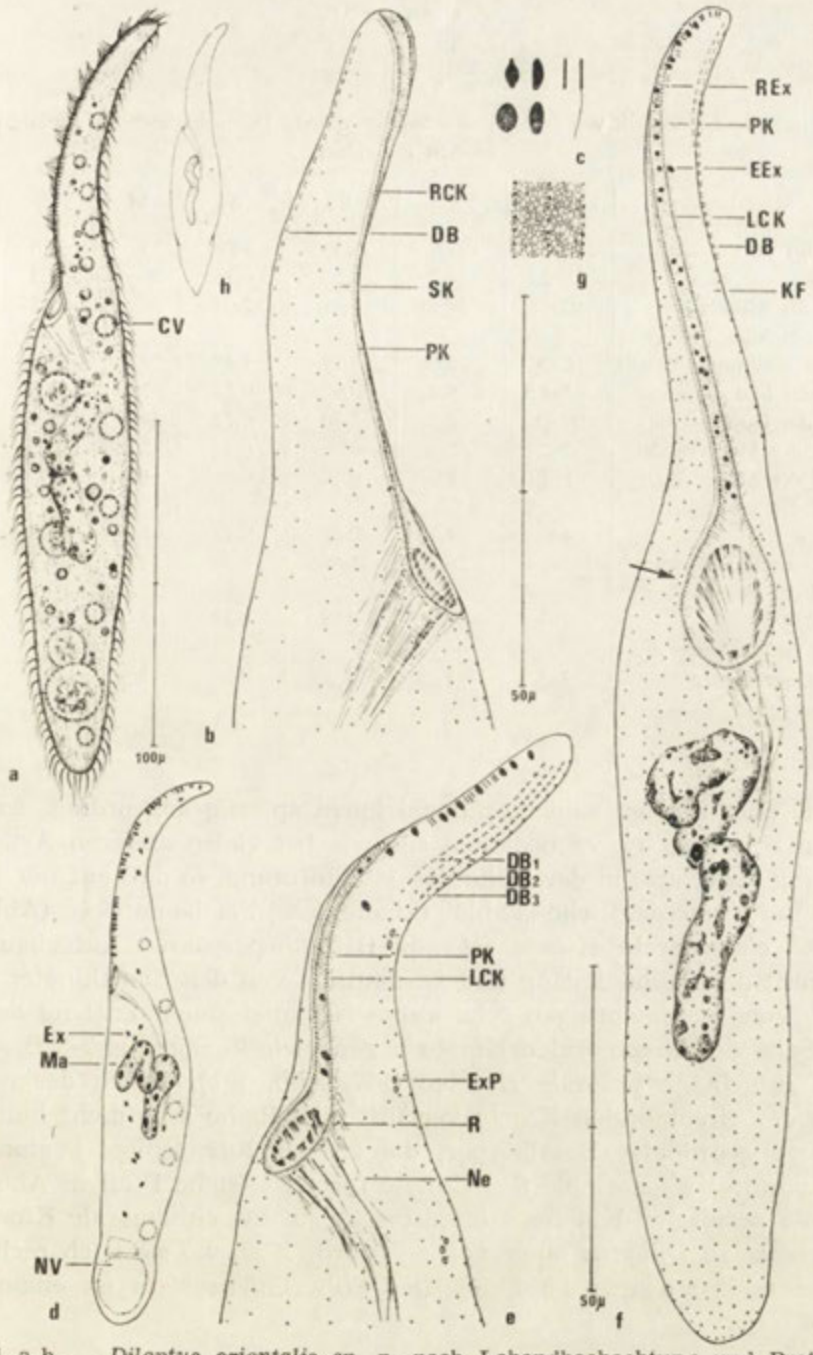


Abb. 1 a-h — *Dileptus orientalis* sp. n. nach Lebendbeobachtung und Protargol-Trocken-Silberimprägnation, a, h — links laterale Ansicht nach Lebendbeobachtungen, b, e — Infraciliatur der rechten und linken Seiten in der anterioren Körperregion, c — ruhende und explodierte Extrusome, d — links laterale Ansicht nach Protargolimprägnation, dargestellt sind Kern, kontraktile Vakuole und Extrusome, f — Infraciliatur der Ventralseite, g — Silberliniensystem in der mittleren Körperregion

Tabelle 1

Biometrische Charakteristik von *Dileptus orientalis* sp. n. Alle Daten basieren auf protargolimprägnerten Individuen

| Merkmal | Extrem. | $\bar{x}$ | $S_{\bar{x}}$ | $S_{\bar{x}}^{-}$ | M | V | n |
|---|---|---|---|---|---|---|---|
| Länge (in µm) | 153–225 | 191.6 | 22.30 | 4.86 | 189 | 11.6 | 20 |
| Breite | 18–23 | 20.7 | 1.52 | 0.36 | 20 | 7.3 | 20 |
| Länge des Rüssels | 50–74 | 58.5 | 7.41 | 2.04 | 58 | 13.5 | 20 |
| Länge des Makronukleus (jeder Makronukleusteil) | 18–28 | 25.6 | 5.53 | 1.48 | 23.5 | 21.6 | 17 |
| Breite des Makronukleus | 5–8.5 | 6.9 | 0.93 | 0.23 | 7.2 | 13.5 | 17 |
| Länge der Dorsalbürste | 20–28 | 24.5 | 2.70 | 0.72 | 24 | 11.4 | 15 |
| Anzahl der Makronuklei | 2–2 | 2 | 0 | 0 | 2 | 0 | 20 |
| Anzahl der Mikronuklei | 1–1 | 1 | 0 | 0 | 1 | | 20 |
| Anzahl der postoralen Kineten | 4–6 | 4.75 | 0.61 | 0.12 | 5 | 12.8 | 24 |
| Anzahl der Somakineten | 15–19 | 16.7 | 1.08 | 0.24 | 17 | 6.5 | 20 |
| Anzahl der Basalkörper in 10 µm | 4–7 | 5.58 | 0.88 | 0.18 | 5 | 15.8 | 24 |
| Anzahl der Reihen der Dorsalbürste | 3–3 | 3 | 0 | 0 | 3 | 0 | 11 |

### Infraciliatur

Die Somakineten sind meridional leicht spiralig angeordnet. Auf der linken Seite des Tieres beginnen sie, wie bei vielen anderen Arten der Gattung auch, erst in der Höhe der Mundöffnung, so daß auf der linken Rüsselseite eine deutliche „kahle" (kinetenlose) Fläche entsteht (Abb. 1 e).

Die circumorale Kinete ist aus Basalkörperpaaren aufgebaut. Sie umgreift den Mundeingang und ist deutlich von den Somakineten abgesetzt. Von ihr entspringen sehr kurze Nematodesmen. Entlang der linken Seite der circumoralen Kinete liegen viele kleine, aus 2-3 Basalkörpern aufgebaute präorale Kineten (PK, Abb. 1 e). Neben der rechten Seite der circumoralen Kinete verläuft eine Reihe sehr dicht hintereinander angeordneter Basalkörper, die an der Rüsselspitze beginnt und sich postoral als normale Somakinete fortsetzt (siehe Pfeil in Abb. 1 f). Die 4-6 postoralen Kineten treffen direkt auf die circumorale Kinete, im Gegensatz zu *Dileptus mucronatus* Penard, 1922, wo sie nach rechts gebogen sind (Foissner 1984). Das Silberliniensystem ist engmaschig (Abb. 1 g).

## Diskussion

Das Problem der Artabgrenzung innerhalb der Gattung *Dileptus* stellt sich durch die Konstanz bzw. die Inkonstanz der Merkmale, die zur Artbestimmung herangezogen werden. Jankowski (1967) hat die

Tabelle 2

Gegenüberstellung ähnlicher Arten der Gattungen *Dileptus* und *Dimacrocaryon*

| | Anzahl der C.V. | Ma-Teile | Mi | Länge des Körpers in µm | Anzahl der SK | Habitat | Form des Hinterendes | Zoochlorellen |
|---|---|---|---|---|---|---|---|---|
| *Dileptus americanus* Kahl. 1935 | 2 | 2 | 1 | 190–220 | ? | Moosrasen | breit gerundet | ohne |
| *Dimacrocaryon amphileptoides* (Kahl, 1931)* | mehrere | 1–2 | 1 | 145–250 | 22–30 | Boden, Moos | breit gerundet | ohne |
| *Dileptus anguillula* Kahl, 1931* | mehrere | 2–11 | 1 | 60–116 | 8–12 | Boden, Moos | breit gerundet | ohne |
| *Dileptus binucleatus* Kahl, 1931 | 2 | 2 | 1 | 300–400 | ? | Salzwasser | leicht zugespitzt | ohne |
| *Dileptus bivacuolatus* de Cunha, 1915 | 2 | 2 | 2 | 100–150 | ? | Süßwasser | leicht zugespitzt | ohne |
| *Dileptus conspicuus* Kahl, 1931 | 2 | 2–4 | 1–2 | 200 | ? | Moosrasen | spindelförmig oder oval | ohne |
| *Dileptus gabonensis* Dragesco, 1963 | 1 | 2 | 1 | 300 | ? | Sand | lang zugespitzt | ohne |
| *Dileptus lacazei* (Gourr et Roes, 1886) | 1 | 2 | 1 | 180–290 | ? | Meerwasser, Salzwasser | zugespitzt | ohne |
| *Dileptus maronensis* Dragesco, 1963 | 2–4 | 2 | 1 | 300 | ? | Sand | zugespitzt | vorhanden |
| *Dileptus mucronatus* Penard, 1922* | mehrere | 1–2 | 1 | 300–500 | 21–30 | Süßwasser, Boden | zugespitzt | ohne |
| *Dileptus terrenus* Foissner, 1981* | ca 10 | 1 | 1 | 152–350 | 26–27 | Boden | zugespitzt | ohne |
| *Dileptus orientalis* sp. n. | 5–8 | 2 | 1 | 150–250 | 15–19 | Boden | breit gerundet | ohne |

* Angaben nach Foissner (1984)

Gattung *Dileptus* nur nach der Form des Makronucleus in 3 Genera (bzw. Subgenera) unterteilt.

Dem stimmt Foissner (1984) nicht zu, da dieses Merkmal nicht für alle Arten konstant ist. Bei *Dileptus orientalis* sp. n. erweist sich die Anzahl der Makronucleus-Teile als sehr konstant (Tab. 1).

Weitere wichtige Merkmale zur Artbestimmung sind die Individualgröße und die Anzahl der Somakineten. Drzewińska und Golińska (1987) haben herausgefunden, daß bei *Dileptus margaritifer* die Individuengröße und Anzahl der Somakineten u.a. vom Ernährungszustand der Tiere abhängt. *Dileptus orientalis-Individuen* sovohl aus den Versuchsansätzen als auch aus Reinkulturen besitzen konstante Größe und Anzahl der Somakineten (Tab. 2).

Es zeigt sich also, daß bei einigen *Dileptus*-Arten Merkmale stark variieren können, die bei anderen jedoch relativ konstant sind (Foissner 1984, Dragesco 1963).

In den bisher veröffentlichten Arbeiten über *Dileptus*-Arten haben die Extrusome (hier: Toxicysten) keine oder wenig Beachtung gefunden. Nach unserer Meinung aber kommt der Form und Größe der Toxicysten durchaus eine Bedeutung zur Artabgrenzung zu.

Summary

The morphology and infraciliature of *Dileptus orientalis* sp. n. from the edaphon of place in Qingdao, P. R. China, is described. *Dileptus orientalis* sp. n. is characterized by the following distinctive marks: the infraciliature consists of 15-19 somatic cineties with a dorsal brush of three rows. The toxicysts are species-characteristic: very short and spindle-shaped. The number of contractile vacuoles varies from 5 to 8, they extend to the top of the proboscis. The posterior end is broadly rounded or bluntly pointed. The size ranges between 150-250 μm. The macronucleus consists of two parts with a micronucleus between them.

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# Observations sur l'infraciliature de *Plagiopyla nasuta* Stein, 1860

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*Synopsis.* Nous avons étudié l'infraciliature somatique et buccale chez le cilié trichostome *Plagiopyla nasuta*. Ce cilié possède un très grand système sécant localisé dans la moitié postérieure de la face droite de la cellule. Deux autres systèmes sécantes sont placés dans le pôle postérieur du cilié. L'infraciliature buccale est constituée par deux lèvres vestibulaires (inférieure et supérieure) dont cinéties ont deux parties différenciées. Les cinéties du bord gauche des deux lèvres forment un petit entonnoir vestibulaire. Cet entonnoir est précédant au cytostome du cilié.

Le genre *Plagiopyla* Stein, 1860 comprend des endosymbiontes et des espèces libres vivantes dans les eaux douces, saumâtres et salées. La morphologie des espèces appartenant à ce genre a fait l'objet de plusieurs études (Kahl 1931, Jankowski 1964, Borror 1972, Dragesco 1972, Agamaliev 1978, Berger et Lynn 1984, Dragesco et Dragesco-Kerneis 1986). Malgré cela l'infraciliature somatique de *Plagiopyla* n'est pas connue entièrement et leur infraciliature buccale reste encore à décrire. D'autre part, pendant les dernières années la position systématique du genre *Plagiopyla* a fait l'objet des discussions (Berger et Lynn 1984, Lee et al. 1985, de Puytorac et al. 1987) ayant compte de certaines particularités de son infraciliature somatique. Dans ce travail nous présentons des données nouvelles concernant l'infraciliature somatique et buccale de *Plagiopyla nasuta*, données qui à notre avis pourraient contribuer à une meilleure connaissance de ce genre.

## Matériel et techniques

Le cilié ayant servi à cette étude, *Plagiopyla nasuta*, a été recolté dans le sédiment de fond d'une lagune d'eau douce non pas poluée, située, au bord de la rivière Henares (Guadalajara, Espagne) dont caractéristiques chimiques sont montrées dans le tableau 1.

L'étude du cilié a été realisé avec la technique d'imprégnation à l'argent de Fernández-Galiano (1976).

Tableau 1

Caractéristiques physique-chimiques de l'eau de la lagune (les analyses ont été effectués selon les techniques de Standard Methods 1985)

| Paramètre | M | m | $\bar{x}$ |
|---|---|---|---|
| Oxygen dissous (mg/l) | 12.2 | 5.2 | 7.8 |
| Temperature (°C) | 22.5 | 5.2 | 12.3 |
| pH | 7.8 | 7.5 | 7.6 |
| Conductivité (μS/cm 25 °C) | 1375 | 761 | 1032 |
| Résidu s (mg/l) 110 °C | 881 | 475 | 665 |
| $NO_3^-$ (mg/l) | 2.7 | 0.04 | 0.82 |
| $NO_2^-$ (μg/l) | 7.0 | 0.0 | 2.0 |
| $NH_4^+$ (μg/l) | 218.0 | 35.0 | 120.0 |
| NTK (mg/l) | 3.1 | 1.1 | 1.8 |
| DQO ($MnO_4^-$) (mg $O_2$/l) | 3.8 | 1.1 | 2.2 |
| P-Phosphates (μg/l) | 70.0 | 20.0 | 39.0 |
| P-Total (μg/l) | 380.0 | 48.0 | 188.0 |
| $HCO_3^-$ (mg/l) | 235.1 | 170.2 | 202.4 |
| $Cl^-$ (mg/l) | 180.9 | 62.4 | 120.0 |
| $SO_4^=$ (mg/l) | 240.0 | 112.0 | 163.2 |
| $SiO_2$ (mg/l) | 13.2 | 6.3 | 9.4 |
| $Ca^{++}$ (mg/l) | 137.6 | 81.6 | 103.4 |
| $Mg^{++}$ (mg/l) | 32.1 | 19.4 | 25.2 |
| $Na^+$ (mg/l) | 112.0 | 45.0 | 70.0 |
| $K^+$ (mg/l) | 4.3 | 2.6 | 3.2 |

M – maximum, m – minimum, $\bar{x}$ – moyenne

## Résultats

### Morphologie générale

*Plagiopyla nasuta* est un cilié de forme ovoïde, aplati latéralement aux dimensions (après fixation avec des vapeurs de $OsO_4$) 88-114 μm

pour la longueur et 44-66 μm pour la largeur. Le tableau 2 montre les données biométriques de cette espèce.

Le macronucleus, généralement ovale, mais parfois esphérique ou

Tableau 2

Données biométriques de *Plagiopyla nasuta* (les mesures ont été realisées avec des vapeurs de $OsO_4$)

| Caractère (μm) | m | M | $\bar{x}$ | Md | s | $s_{\bar{x}}$ | cv | n |
|---|---|---|---|---|---|---|---|---|
| Longueur totale | 88.0 | 114.4 | 101.9 | 95.1 | 8.0 | 1.6 | 7.9 | 25 |
| Largeur totale | 44.0 | 66.0 | 54.2 | 52.8 | 5.9 | 1.2 | 10.8 | 25 |
| Longueur du macronucleus | 24.2 | 33.0 | 29.2 | 28.6 | 2.5 | 0.5 | 8.4 | 25 |
| Largeur du macronucleus | 17.6 | 24.2 | 20.6 | 20.9 | 1.6 | 0.3 | 7.7 | 25 |

m – minimum, M – maximum, $\bar{x}$ – moyenne, Md – médiane, s – déviation standard, $s_{\bar{x}}$ – erreur standard, cv – coefficient de variation, n – nombre d'individus

irrégulier, est situé dans la partie antérieure de la cellule. Accolé à lui il y a un micronucleus très petit et très difficile d'observer.

L'ouverture vestibulaire est une étroite gouttière transversale localisée dans le tiers antérieur du cilié (Pl. I 1,2, Fig. 1).

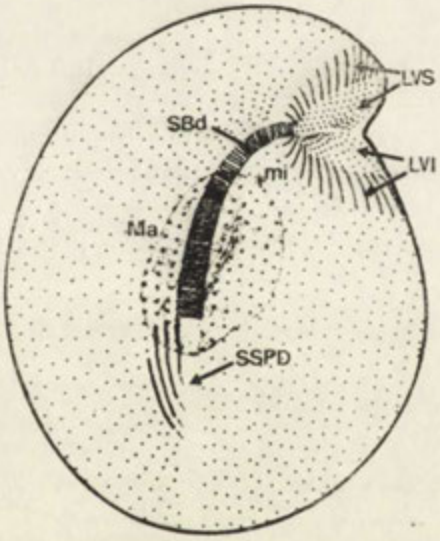


Fig. 1 Schème de la face droite de *Plagiopyla nasuta*. LVI — lèvre vestibulaire inférieure, LVS — lèvre vestibulaire supérieure, Ma — macronucleus, mi — micronucleus, SBd — bande striée, SSPD — système sécant postérieur droit

## Infraciliature somatique

*Plagiopyla nasuta* possède 80-90 cinéties somatiques constituées par des cinétosomes isolés (monocinétides au sens de Lynn et Small 1981) qui possèdent des fibres cinétodesmales très longues, atteignant le cinétosome antérieur. A gauche et en arrière de chaque cinétosome,

on observe un sac parasomal (Pl. I 3). D'ailleurs, dans les espaces intercinétiques existent des structures argyrophiles régulièrement distribuées qui, par son aspect, pourraient être des trichocystes (Pl. I 3).

Dans la face droite du cilié, une bande striée (d'environ 3 μm de largeur) s'étend longitudinalement dans les deux tiers antérieures de la cellule (Pl. I 1, Fig. 1). Cette bande comprend un espace intercinétique plus grand que celui correspondant aux restantes cinéties somatiques. Dans cet espace on observe des striatons transversales très faibles (Pl. I 4). Les cinéties limitant à gauche et à droite la bande striée possèdent des cinétosomes très proches et moins argyrophyles que ceux qui constituent les cinéties somatiques restantes (Pl. I 4).

La bande striée continue vers le pôle antapical du cilié par un grand système sécant postérieur droit (Pl. I 1,5, Fig. 1) qui s'étend à peu près dans toute la moitié postérieure de la cellule. Ce système est constitué par 6-10 cinéties dont les 2-3 prémières ont à son extrémité postérieure des cinétosomes très serrés et très argyrophyles (Pl. I 5, Fig. 1).

Nous avons observé aussi que dans le pôle postérieur de *P. nasuta* il y a deux systèmes sécantes antapicales: dorsal et ventral qui convergent avec le système sécant postérieur droit déjà décrit (Pl. I 6).

## Infraciliature buccale

Toutes les cinéties somatiques convergent dans le tiers antérieur de la cellule y constituant deux lèvres vestibulaires: inférieure et supérieure (Pl. II 7, 9, Fig. 2).

Les cinéties ventrales pénétrent directement dans l'ouverture vesti-

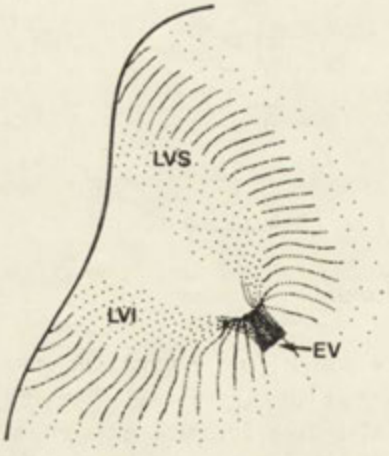


Fig. 2 Schème de l'infraciliature buccale de *Plagiopyla nasuta*. EV — entonnoir vestibulaire, LVI — lèvre vestibulaire inférieure, LVS — lèvre vestibulaire supérieure

bulaire constituant la lèvre inférieure. Par contre, les cinéties dorsales, qui tapissent le pôle apical du cilié, sont interrompues avant atteindre le vestibule, donc il existe une ligne vide précédant la lèvre supérieure (Pl. II 8).

Dans les cinéties des lèvres vestibulaires on peut distinguer deux parties différenciées: (1) la partie éxtérieure avec des cinétosomes serrés entre lesquels on observe une certaine connexion par une structure argyrophyle, et (2) la partie intérieure qui présente des cinétosomes sans connexion et plus écartés que aux de la partie éxtérieure (Pl. II 9, Fig. 2)

Les cinéties vestibulaires vont vers le côté gauche de la cellule (Pl. II 9, Fig. 2) mais seulement celles qui sont situées dans le bord gauche des chacune des deux lèvres pénétrent dans l'entonnoir vestibulaire (Pl. II 10, Fig. 2). L'entonnoir est très difficile à observer tant par sa petite taille que par sa localisation. Il est précédant au cytostome et à la cytopharynx du cilié (Pl. II 10).

## Discussion

Les dimensions de *Plagiopyla nasuta* que nous avons mesuré coïncident avec celles signalées par Kahl (1931) pour la varieté *wetzeli*, Jankowski (1964), Agamaliev (1978), Dragesco et Dragesco-Kerneis (1986), mais elles sont légérèment supérieures à celles indiquées par Borror (1972). Par contre, le nombre de cinéties somatiques signalé par tous les auteurs pour *P. nasuta* est inférieur à celui que nous avons trouvé pour cette espèce. Le tableau 3 montre les données signalées par les différentes auteurs pour *Plagiopyla nasuta*.

Les cinéties somatiques, tel qui signalent Dragesco et Dragesco-Kerneis (1986), sont constituées par des cinétosomes plus écartés que ceux qui a montré Jankowski (1964), mais il possèdent une très longue fibre cinétodesmale qui atteint le cinétosome antérieur, ce qui peut expliquer l'interprétation donnée par Jankowski.

Nous sommes d'accord avec tous les auteurs en ce qui concerne l'existence d'une bande striée dans la face droite du cilié, mais en plus nous avons trouvé que les cinéties limitant cette bande diffèrent des autres cinéties somatiques. D'ailleurs, nous avons observé que cette bande continue vers le pôle antapical par un grand système sécant postérieur droit qui n'a pas été décrit jusqu'à présent. Seulement Borror (1972) a signalé la présence de trois cinéties plus argyrophyles à la suite de la bande striée, lesquelles, à notre avis, correspondent aux 2-3 prémières cinéties d'un arrangement décrit par nous. Jankowski (1964) montre

Tableau 3

Caractéristiques de *Plagiopyla nasuta* données par les différentes auteurs

| Auteur | Caractère | | | |
|---|---|---|---|---|
| | Longueur totale (μm) | Largeur totale (μm) | Nombre de cinéties | Forme du macronucleus |
| Kahl (1931) | 100–150 | – | – | Ovoïde |
| Kahl (1931)[1] | 70–90 | – | – | Ovoïde |
| Jankowski (1964) | 90–120 | – | 55–65 | Irrégulier |
| Borror (1972) | 65–95 | 38–57 | – | Ovoïde |
| Agamaliev (1978) | 90–110 | 50–60 | 60–70 | Ovoïde |
| Dragesco (1972) Dragesco et Dragesco-Kerneis (1986) | 80–150 | – | 55–66 | Variable |
| Espagne Sola et al., 1988 | 88–114 | 44–66 | 80–90 | Généralement Ovoïde |

[1] pour *Plagiopyla nasuta* var. *wetzeli*

l'existence d'un système sécant dans la face gauche de la cellule qui peut être, à notre avis, le système que nous avons décrit comme le système sécant antapical ventral.

Tous les auteurs qui ont étudié le genre *Plagiopyla* observent que les cinéties somatiques pénétrent dans le vestibule dans lequel les cinétosomes sont très serrés, mais il ne signalent pas la disposition des cinéties vestibulaires. Chez *Plagiopyla nasuta* les cinéties vestibulaires présentent deux parties différenciées lesquelles, à notre avis, pourraient être l'equivalent des parties supraorale et orale trouvées par Berger et Lynn (1984) chez le cilié trichostome *Lechriopyla mystax*.

Selon nos observations, les cinétosomes de la partie éxtérieure des lèvres vestibulaires montrent une connexion par une structure argyrophyle. Celle-ci pourrait correspondre au rideau microtubulaire décrit par Berger et Lynn (1984) chez *Lechriopyla mystax*, où d'autre part, elle peut être une fibre cinétodesmale comme celle qui a été trouvée dans le vestibule de quelques trichostomes (Grain 1966).

Nous avons observé un petit entonnoir constitué par les cinéties vestibulaires du bord gauche des deux lèvres. Cet entonnoir, jamais décrit jusqu'à présent est précédant au cytostome et à la cytopharynx du cilié.

En ce qui concerne la position systématique du genre *Plagiopyla*, Berger et Lynn (1984) ont suggéré, que selon les caractéristiques du

cortex somatique, *Plagiopyla* doit être inclu parmi les ciliés de la Classe *Oligohymenophorea*. Nous pensons, en accord avec de Puytorac et al. (1987), que malgré la présence de fibres cinétodesmales très longues, comme celles décrites chez certaines ciliés de la Classe *Oligohymenophorea*, l'infraciliature buccale de *Plagiopyla nasuta* ressemble plutôt celle qu'on a trouvé chez les espèces provenant de l'Ordre *Trichostomatida* de la Classe *Vestibuliferea*, et c'est pour ça que nous croyons que le genre *Plagiopyla* doit rester y inclu.

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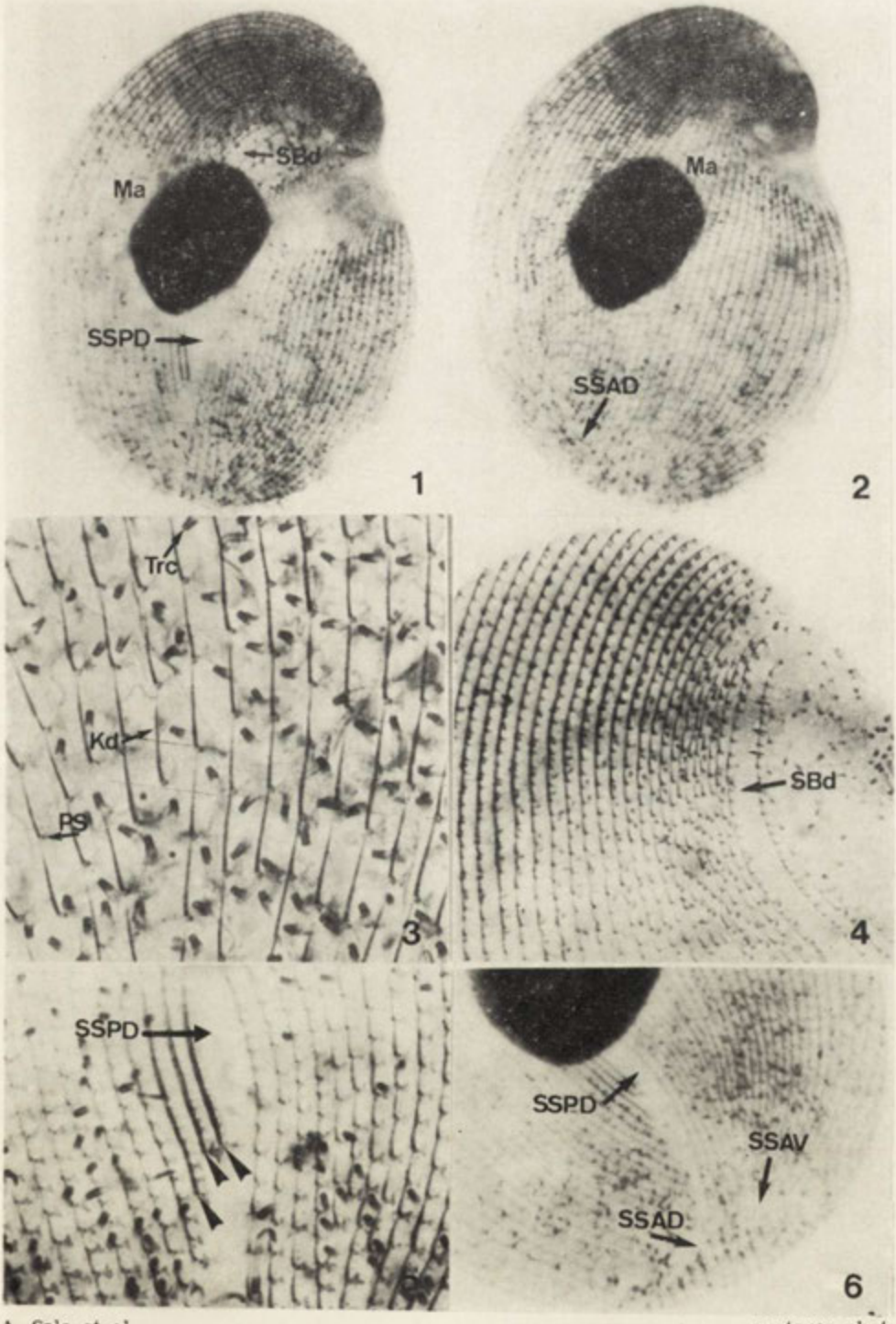
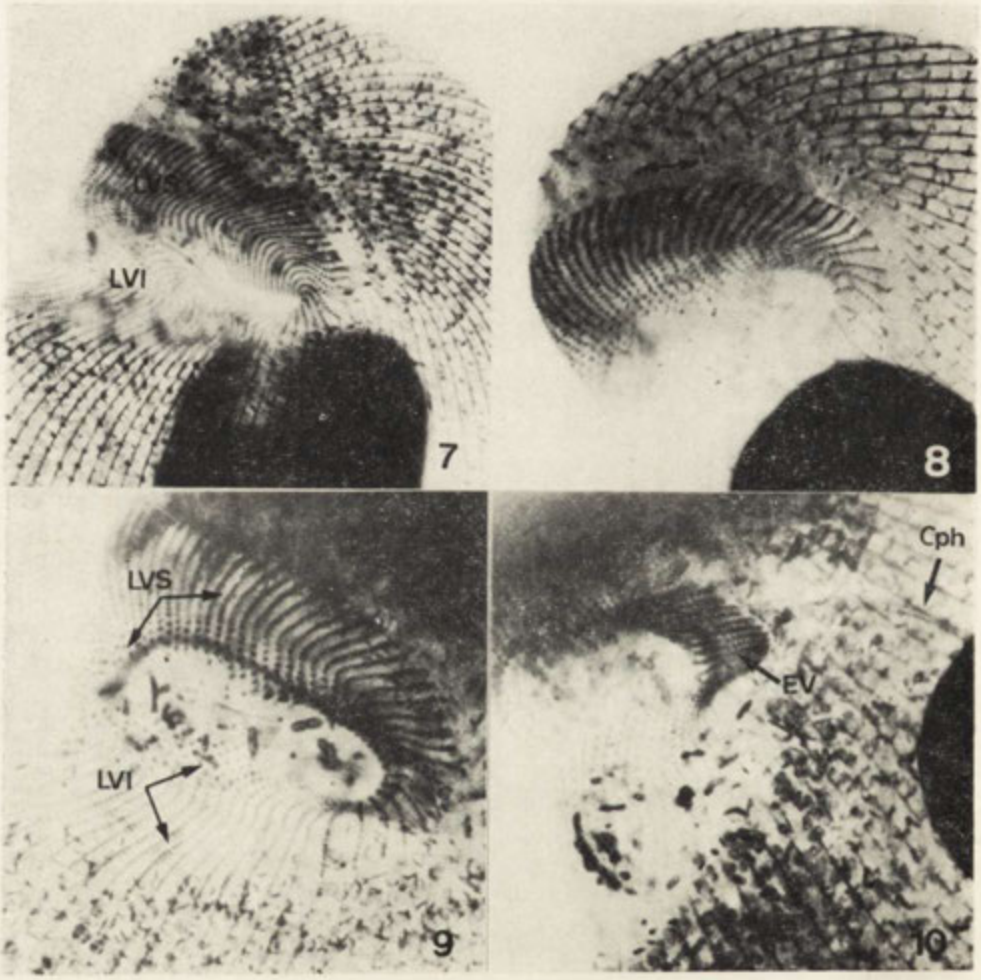
EXPLICATIONS DE PLANCHES I-II

1: Vue générale de *Plagiopyla nasuta*: face droite du cilié (×450). Ma — macronucleus, SBd — bande striée, SSPD — système sécant postérieur droit
2: Face gauche de *P. nasuta* (×450). Ma — macronucleus, SSAD — système sécant antapical dorsal
3: Détail de l'infraciliature somatique (×1850). Kd — fibre cinétodesmale, PS — sac parasomal, Trc — trichocystes
4: Détail des striations transversales (petites filèches) de la bande striée (SBd) (×950)
5: Détail des prémières cinéties (pointes de flèches) du système sécant postérieur droit (SSPD) (×1300)
6: Pôle postérieur du cilié: systèmes sécantes antapicales dorsal (SSAD) et ventral (SSAV), et sa convergence avec le système sécant postérieur droit (SSPD) (×550)
7: Infraciliature buccale de *P. nasuta*: lèvre vestibulaire inférieure (LVI) et lèvre vestibulaire supérieure (LVS) (×650)
8: Pôle apical du cilié: ligne vide (double flèche) précédant la lèvre supérieure (×850)
9: Détail des cinéties des deux lèvres vestibulaires (LVI et LVS) dans lesquelles on peut distinguer deux parties (flèches) (×1100)
10. Détail du parcours des cinéties vestibulaires et de l'entonnoir vestibulaire (EV) (×1000) Cph-cytopharynx




A. Sola et al. auctores phot.

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# *Crithidia bombi* sp. n. a Flagellated Parasite of a Bumble-bee *Bombus terrestris* L. (*Hymenoptera, Apidae*)

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*Received on* 4th January 1988

*Synopsis. Crithidia bombi* sp. n. is edescribed from one of two wild populations of *Bombus terrestris* L. in Italy. The parasite inhabits the gut of adult workers but its pathological effect on the host is not clear. In one population of *B. terrestris* infection during June reached level of 14%.

During a short survey of pathogens of various insects conducted in Southern Italy we recorded for the first time a flagellate infection in *Bombus terrestris* L. which we report in this paper.

## Material and Methods

Adult workers of *Bombus terrestris* L. were collected by sweeping net in Policoro (cultivated fields) and in Bari (University Campus). Insects were anaesthetized, dissected and their hemolymph, gut and other tissues were microscopically examined at magnification 400× or 1000×.

Smeared preparations of the tissues or the gut contents were fixed in methanol for 2 min and stained in 0,25% Giemsa's solution for 16 h.

The holotype slide is deposited in the collection of the senior author.

## Results

### Infection Level

Out of 34 workers of *B. terrestris* collected in Policoro 5 insects (14.7%) were infected by a flagellate while 14 adults collected in Bari did not harbor this parasite.

Morphology

The flagellate inhabits the gut and two morphological forms are observed: choanomastigote and amastigote.

The observed motile choanomastigotes had the maximum length 8.1 μm (Pl. I 1,2). Their bodies are pear-like in shape and their anterior end is truncated as here the large reservoir opens. The nucleus is oval, has the diameter of 1.86 μm and is located centrally or closer to the posterior end. The bean-shaped kinetoplast is about 0.8-1.0 μm long and is located close to nucleus in the anterior part of the body. The flagellum starting from kinetosome is thin, 8-12 μm long and emerges from wide funnel-shaped reservoir.

The amastigotes — which are as numerous as choanomastigotes — are round, oval or pear-like shaped with the diameter dimensions 4.9 μm (Pl. I 1,2). The nucleus of amastigote forms is oval, about 1.60 μm in diameter, and located laterally or at the posterior end of the body. The kinetoplast closely adjacent to the nucleus is located often between the nucleus and the body wall opposite to the large funnel-shaped reservoir (Pl. I 2). The flagellum is mostly absent or if present it is very short and does not emerge from the reservoir.

Taxonomic Position

This is the first record of a flagellate infection in bumble-bees *Bombus* spp. However, in a honey-bee *Apis mellifica* L., belonging to the same family *Apidae*, flagellate infections are known and frequently observed (Borchert 1966; Wallace 1966). Lotmar (1946) described *Leptomonas* apis while Langridge (1966) and Lom (1964) recorded *Crithidia* sp. which was later described as *Crithidia mellificae* by Langridge and McGhee (1967).

The flagellate recorded in *Bombus terrestris* belongs to genus *Crithidia* characterized by forms, having a pear-like body (short and wide) and a very characteristic funnel-shaped reservoir (Haare and Wallace 1966; Wallace 1963).

The comparison of morphological features of flagellates known from *Apidae* family (Table 1) indicates that choanomastigote forms of *Crithidia* recorded in *B. terrestris* differ significantly by their smaller size from *C. mellificae* and shorter flagellum. It is therefore concluded that *Crithidia* recorded in *B. terrestris* is a new species and a name *Crithidia bombi* sp. n. is proposed.

The character of pathogenicity of *C. bombi* sp. n. to its host *B. terrestris* is not clear and requires to be studied as well as epizootiological problems and host range.

In case of *Apis mellifica* such authors like Bahrman (1965), Bor-

Table 1

Comparison of measurements of trypanosomatids recorded in *Apidae* (*Hymenoptera*)

| Species | Host | Size in μm | |
|---|---|---|---|
| | | body | flagellum |
| *Leptomonas apis* (Lotmar 1946) | *Apis mellifica* L. | 20–25 | not given |
| *Crithidia* sp. (Lom 1964) | *Apis mellifica* L. | 7–11×2–4 | 16–20 |
| *Crithidia mellificae* (Langridge and McGhee 1967) | *Apis mellifica* L. | 3.41–10.85×2.87–8.98 | not given |
| *Crithidia bombi* sp. n. | *Bombus terrestris* L. | 4.9–6.9×1.5–2.4[1]<br>3.4–5.4×3.4–5.4[2] | 8–12 |

Legends: [1] choanomastigote stage; [2] amastigote stage

chert.(1966), Fyg (1954), Giavarini (1956), Grobov et al. (1987), Hischier (1962) and Kluge (1963) report that flagellate infection among workers of *A. mellifica* are quite common in spring and summer but in winter infection is hardly recorded. The reasons for this are not clear and nutritional or temperature factors may play a role in this phenomenon.

## ACKNOWLEDGEMENT

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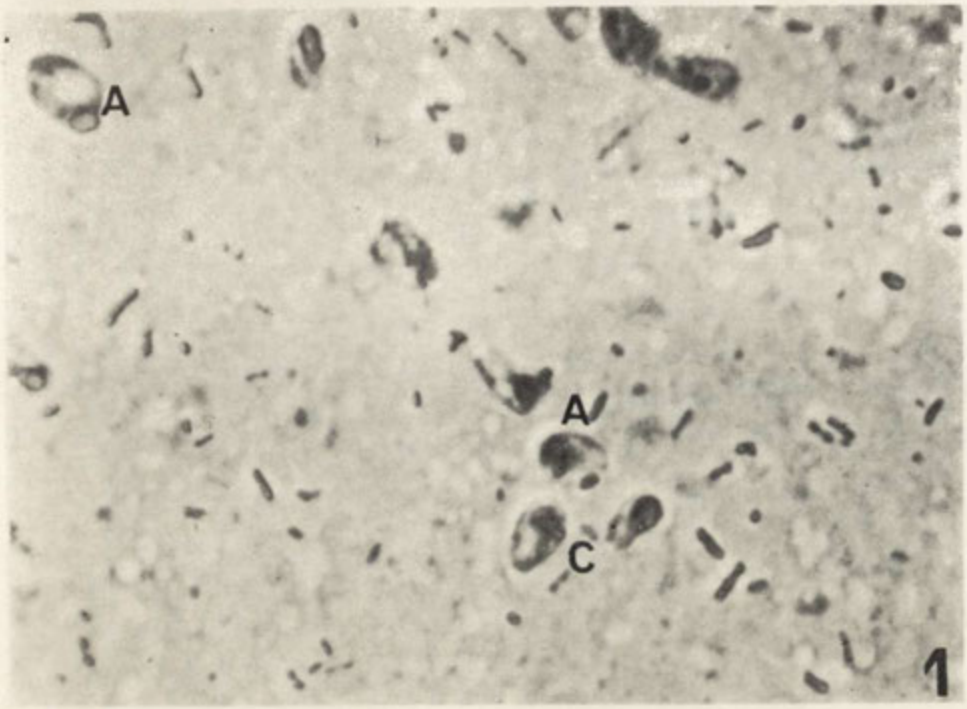
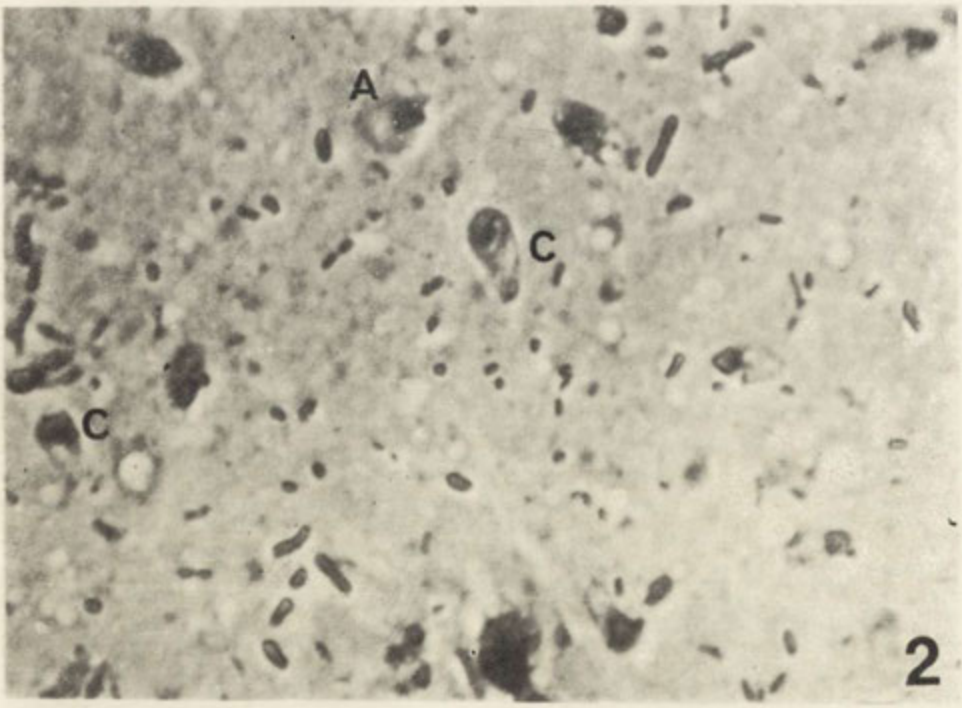
## EXPLANATION OF PLATE

*Crithidia bombi* sp. n.

1-2: Choanomastigote (C) and amastigote (A) forms in smeared and stained gut contents of *Bombus terrestris*




J. J. Lipa and O. Triggiani auctores phot.

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# *Lecudina capensis* sp. n. Parasitic Gregarine of *Pherusa laevis* Stimpson, 1856 (Polychaete Annelid)

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*Received on* 15 May 1987, *revised on* 19 January 1988

*Synopsis.* The morphology of *Lecudina capensis* sp. n. parasitic in *Pherus laevis* Stimpson, 1856 digestive tract, a polychaete found in *Laminaria* rhizomes, located on the continental platform of Namibia (South Africa) has been described. The results showed a great polymorphism of the trophozoites and the sporozoites have length of 75 μm. The elongate sporadines are the most abundant form, and their length is 120-130 μm. Cephalins have one fixation apparatus of 25 μm long. The gamonts have navicular form and they have a longitudinal crest and fine folds in the surface, with average sizes of 215 μm and lateral syzygy. The spherical sporozoites have been observed. Spores were not seen.

## Material and Methods

The gregarine were separated from the digestive tract of the polychaete *Pherusa laevis* Stimpson, 1856, which have been removed from *Laminaria* rhizomes. These were pulled up from the seafloor and taken to the ship by the drag fishing nets used in the capture of commercial fish species.

The study area was located between 26° S latitude and 14° E longitude, and corresponded to the continental platform of Namibia (South Africa). The work was carried out on board of the freezer ships "Mar del Cabo" and "Egzuki" (Fig. 1).

The average depth was approximately 150-210 braces. The sediment was of fine grained muds. The temperature ranged between 7 and 11°C.

After separating the polychates from the rhizomes they were placed in a container, where after a few days they were anaesthetized by adding water with some crystals of $Cl_2Mg$. After 20 min dissection was carried out.

When dissecting the animal, almost the whole digestive tract is found in the anterior part, and there are loops increasing the surface of food absorption. Four areas can be distinguished morphologically (Fig. 2). First we found a short pharynx,

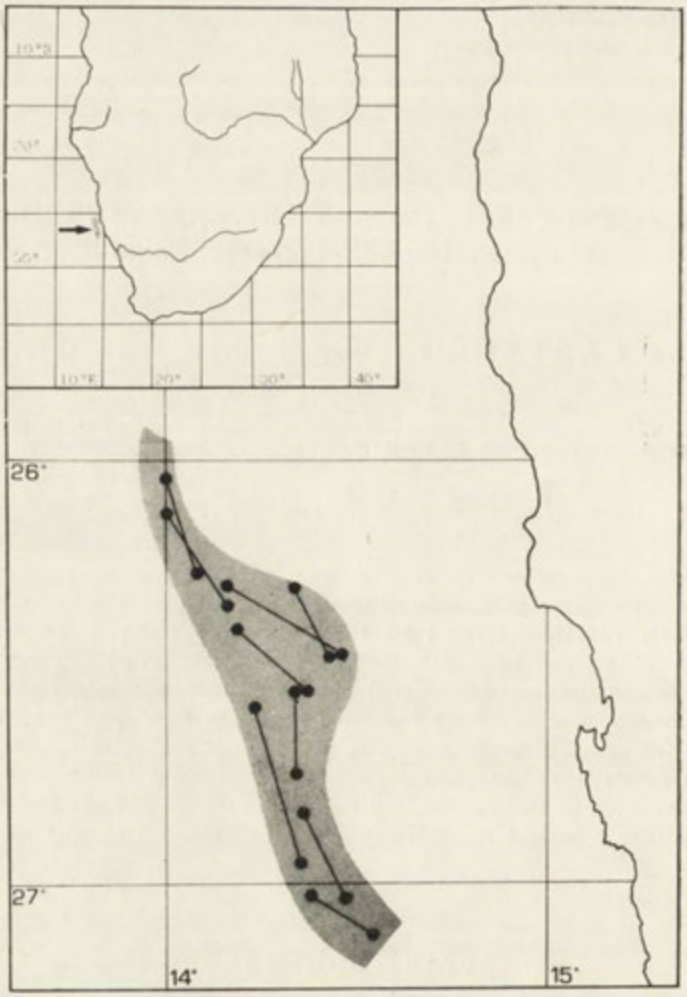


Fig. 1. Map of South Africa. The sampling area is indicated by the arrow

followed by the stomach characteristically bright red in living organisms. Then the wall lining thickens to form what we call the anterior intestine, which loops around the stomach, progressing first in a forward direction, and later towards the "tail" where the wall linning decreases in width and the posterior intestine is found.

The digestive tract was extracted and washed in salt water. Then it was sliced longitudinally also fractionated by parts so as to determine the location of the different gregarine forms. Parasites were only found in the stomach and the anterior intestine.

Formalin 40% and Bouin liquid were used as fixatives and glutaraldehyde in phosphate buffer when destined for observation with the electron microscope. For *in toto* observations the organisms were stained with groad hematoxylin and then were mounted in Hoyer liquid in gelatinized glycerine following the method of Kaiser.

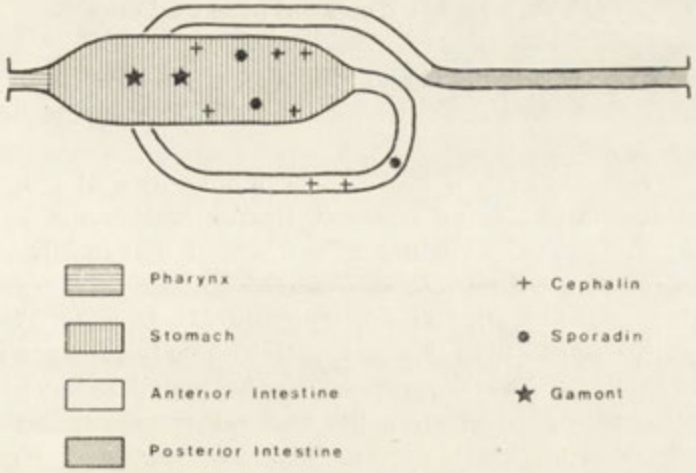


Fig. 2. Outline of the digestive tract

The cytometric measurements of the gregarine were taken of living organisms, of photographs and on microscopic slides. A total of eight parameters were measured: total length (tl), anterior length (al), nucleus width (nw), nucleus length (nl), maximum width (mw), height of the trophozoite (h), height of the crest (hc), fixation apparatus length (el). (Fig. 3).

The determination of the polychaetes has been carried out with the help of "A Monograph of the Polychaeta of Southern Africa", D a y (1967).

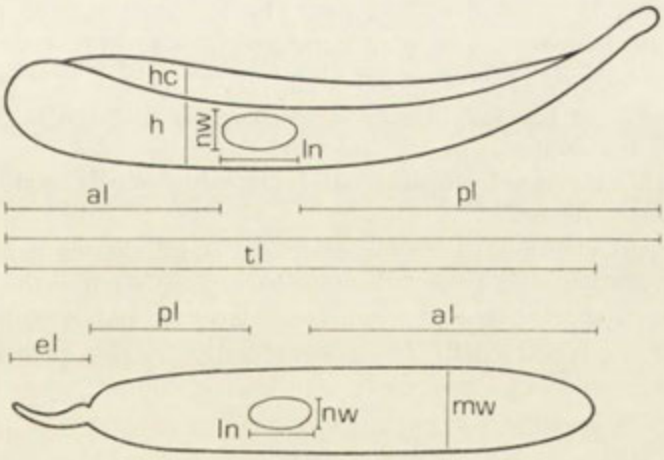


Fig. 3. Measurements taken of the trophozoites: h — height, hc — height of the crest, nw — nucleus width, mw — maximum width, al — anterior length, tl — total length, el — fixation apparatus length, ln — length of the nucleus, pl — posterior length

## Observations and Results

Different stages of life-cycle of this gregarine have been observed and we believe that the characteristics are such as to enable its designation as a new species.

The sporozoites. All share the common trais of a hyaline cytoplasm and size 75 μm. In all of them, flexing movements were observed, but they are not very vigorous. They were found in large numbers, 30-40, although not in all polychates observed.

The cephalins are around 190 μm long and 12 μm wide. The nucleus (29 μm) is very close to the intestinal wall. The body presents longitudinal folds (Pl. I 3). In those cases in which the cephalins have been seen (Pl. I 1) with one end penetrating the host cell, some organelles, which were stained red with the Mallory method, were detected. We associate these organelles with the roptrias, or anterior dense bodies, attributed with the job of perforation of the cell wall of the host (Pl. I 2).

In some preparations the cephalins were successfully removed without breaking the fixation apparatus (epimerite?) (Pl. II 5), and it was observed to measure 25 μm in length and 5 μm wide at the base.

The sporadins are the most abundant forms observed (73%) and can be found both in the stomach and in the anterior intestine. They are elongated and their maximum length is 220 μm, although they usually range between 120 and 130 μm. The mark of where the fixation apparatus has come off can be seen in the anterior part (Pl. I 4).

The nucleus, in the central position, has only one karyosome (Pl. II 6). It measures between 8 and 14 μm. The area anterior to the nucleus is clearer and presents a vesicular structure.

In movement, the posterior zone of the cytoplasm is revealed to be more hyaline than the rest. There is also a clear differentiation between the ecto and the endoplasm.

There are superficial longitudinal folds which are clearly seen with the optical microscope.

The gamont, having navicular shape, can be distinguished without any doubt. The caudal area is distinctly separate from the imaginary medial line that traverses the gregarine longitudinally. Following this axis there is a crest which appears suddenly in the anterior area to decrease later in height and disappear in the caudal area (Pl. II 7-8). The maximum height of 9 μm of the crest is attained in the anterior third.

The gamont can be as long as 328 μm, but the average size is 215 μm long, 36 μm wide and 18 μm high.

The nucleus, is located in the first third of the cell and is elongated

in the direction of the axis of symmetry. It measures between 18 and 26 μm in length and 17 to 2 μm in width. Only one karyosome was observed.

Fine folds were observed on the gamont's surface using the scanning microscope (Pl. II 9).

Syzygy was lateral with the gamonts of the same size (Pl. II 10). The sphaerical sporocysts have been observed but not measured. Spores were not seen.

## Discussion

The studies of gregarines parasitic in polychaetes are abundant and references can be found in all groups of those worms. This is not the case, however, of *Pherusa laevis* Stimpson, 1856 and therefore the study of its parasite gregarine is of interest.

The family *Lecudinidae* erected by Kamm (1922), where is included the genus *Lecudina* Mingazzini (1891) studied by Brasil (1909), Mackinnon and Ray (1931) and Ganapati (1946), contains conflictive species of gregarines regarding their determination.

The great polymorphology shown by the species of the genus *Lecudina* in their trophozoite phase, is a well-known fact. This polymorphologic factor, along with the great homogeneity of the sexual reproduction and with the difficulty of distinguishing clearly whether the attachment organ is a mucron or an epimerite, causes great problems in their specific identification. *Lecudina capensis* sp. n. as well as *L. polymorpha* Schrèvel (1963 a, b) shows trophozoites variously shaped, but it differs from this later in its smaller size and the structure of the attachment appendage. The syzygy type is not being known in *L. polymorpha*.

*L. capensis* sp. n. shows elongated trophozoites and similar to those of *L. platynereidis* (Schrèvel 1969 a, b) but in this later the syzygy is frontal while in *L. capensis* is clearly lateral.

Taking into account everything in this paper and although it has been impossible to study the complete cycle of *L. capensis* sp. n. we think that it is a new species because it differs from the other known species of *Lecudina*.

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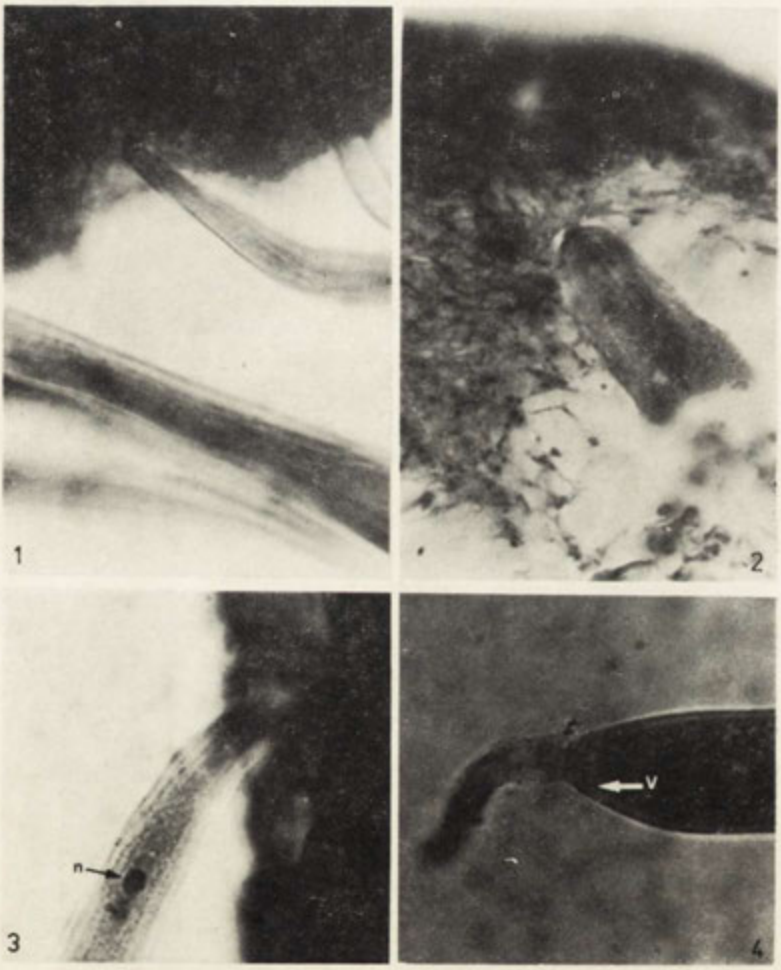
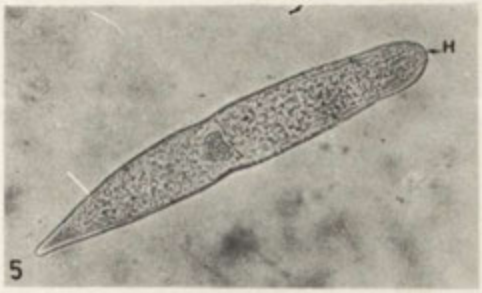
## EXPLANATION OF PLATES I-II

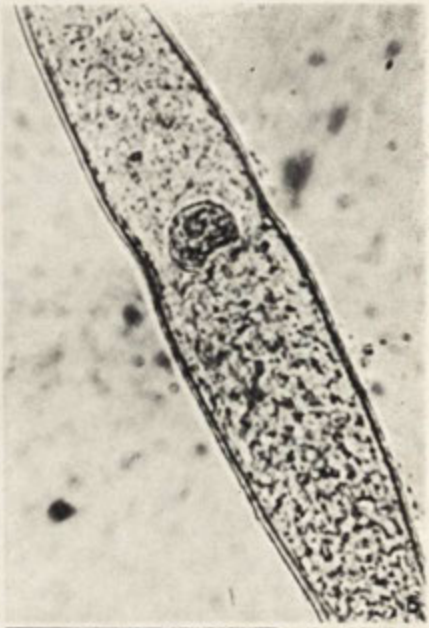
Pl. I 1: Sectation of the stomach of *Pherusa laevis* and area of fixation (1500×)
2: Detail of the area of fixation (1500×)
3: Notice of the superficial folds. N — nucleus (1500×)
4: Cephalin, detail of the area of union of the fixation apparatus (epimerite?) V. (1500×)
Pl. II 5: Sporadine after release, notice the mark of the fixation apparatus (epimerite?). V. (1500×)
6: Nucleus in the central position, and the karyosome 7-8: *Lecudina capensis* sp. n. Gamont, the crest is visible (400×)
9: Scanning electron micrograph of the longitudinal folds in the pellicle of *L. capensis* sp. n. (2100×)
10: *Lecudina capensis* sp. n. syzygy *in vivo*. (100×)



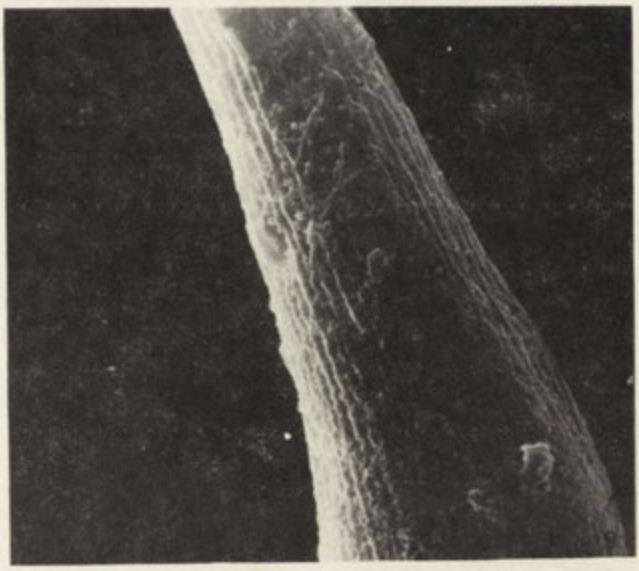

C. Castellón and Mª del Pilar Gracia auctores phot.









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## BOOKS RECEIVED

THE BIOLOGY OF DINOFLAGELLATES

Botanical Monographs, Volume 21
*Edited by* F. J. R. TAYLOR
Blackwell Scientific Publications, Oxford, London, Edinburgh, Boston, Palo Alto, Melbourne, 785 pp.

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*Contributors, Preface*, 1 — General group characteristics, special features of interest, short history of dinoflagellate study (F. J. R. TAYLOR) 2 — Dinoflagellate morphology (F. J. R. TAYLOR), 3 — Dinoflagellate ultrastructure and complex organelles: A. General ultrastructure (J. D. DODGE), B. Complex organelles (C. GREUET), 4 — Biochemistry of the dinoflagellate nucleus (P. J. RIZZO), 5 — Photosynthetic physiology of dinoflagellates (B. PRÉZELIN), 6 — Heterotrophic nutrition (G. GAINES and M. ELBRÄCHTER), 7 — Bioluminescence and circadian rhythms (B. M. SWEENEY), 8 — Dinoflagellate toxins (Y. SHIMIZU), 9 — Dinoflagellate sterols (N. W. WITHERS), 10 — Behaviour in dinoflagellates (M. LEVANDOWSKY and P. KANETA), 11 — Ecology of dinoflagellates: A. General and marine ecosystems (F. J. R. TAYLOR), B. Freshwater ecosystems (U. POLLINGHER), 12 — Dinoflagellates in non-parasitic symbioses (R. K. TRENCH), 13 — Parasitic dinoflagellates (J. and M. CACHON), 14 — Dinoflagellate reproduction (L. A. PFIESTER and D. M. ANDERSON), 15 — Dinoflagellate cysts in ancient and modern sediments (D. K. GOODMAN), Appendix — Taxonomy and classification (F. J. R. TAYLOR), *Taxonomic index, Subject index*

KEY TO PARASITES OF FRESH-WATER FISH OF USSR

*Editor:* O. N. BAUER, Vol. 1, Parasitic Protozoa (ed. S. S. SHULMAN) Keys to Fauna of USSR edited by Zoological Institute of Academy of Sciences of USSR, Leningrad No. 140, Leningrad 1984, pp. 428, 609 figs.

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Introduction, Subregnum Protozoa, Phyllum *Mastigophora*, Class. *Kinetoplastomonada*, Order *Trypanosomamonadida*, Fam. *Trypanosomidae*, Order *Bodomonadida*, Fam. *Bodonidae*, Class. *Parasitomonada*, Order *Proteromonadida*, Fam. *Karatomorphidae*, Order *Diplomonadida*, Phyllum *Sarcodina*, Subphyllum *Lobosa*, Class *Gymnamoebia*, Order *Amoebida*, Fam. *Entamoebidae*, Class *Thecia*, Order *Paramoebida*, Fam. *Mayorellidae* Phyllum *Sporozoa*, Class. *Coccidiomorpha*, Subclass. *Coccidiomorphina*, Order *Coccidiida*, Fam. *Eimeridae*, Order *Adelaida*, Fam. *Adeleidae* Phyllum *Microsporidia*, Class. *Microsporidae*, Order *Glugeida*, Fam. *Glugeidae* and *Thelohaniidae* Phyllum *Cnidosporidia*, Class. *Myxosporidia*, Order *Bivalvulea*, Suborder *Bipolaria*, Fam. *Myxidiidae* and *Neomyxobolidae*, Suborder *Eurysporea*,

Fam.: *Ceratomyxidae*, *Sphaerosporidae* and *Myxobilatidae*, Suborder *Platysporea*, Fam.: *Myxosomatidae*, *Myxobolidae*, Phyllum *Ciliophora*, Class. *Pleurostomata*, Order *Amphileptida*, Fam. *Amphileptidae*, Class. *Cyrtostomata*, Order *Hypostomatida*, Fam. *Chilodenellidae*, Class. *Rimostomata*, Order *Balantidiida*, Fam. *Balantidiidae*, Class. *Hymenostomata*, Order *Tetrahymenida*, Fam. *Tetrahymenidae*, Fam. *Ophryoglenidae*, Class *Suctoria*, Order *Acinetida*, Fam. *Erastophryidae*, Order *Trichophryida*, Fam. *Trichophryidae*, Class. *Peritricha*, Order *Peritrichida*, Suborder *Sessilina*, Fam.: *Scyphidiidae*, *Ellobiophryidae* and *Epistylididae*, Subfam. *Epistylidinae* and *Apiosomatinae*, Fam. *Operculariidae* Suborder *Mobilina*, Fam. *Trichodinidae*

Protozoa incertae sedis. Main compedia, reference books and keys to fish parasites, References, Russian-latin dictionary of fish names. Index of parasites

PROTOZOENFAUNA [FAUNA OF PROTOZOA]

*Editor* Prof. dr Dieter *MATTHES*, Erlangen-Nürnberg
Vol. 7/1, *Suctoria* and *Urceolariidae* (*Peritricha*)
Prof. dr Dieter MATTHES, Erlangen-Nürnberg, Dr Walter GUHL, Düseldorf and Prof. dr Gerhard HAIDER, Stuttgart
Gustav Fischer Verlag, Stuttgart, New York, 1988, XIV, 309 pp., 204 figs., 1 tabl., (in German)

CONTENTS
Preface

*Suctoria*

Introduction, General part: Order *Suctoria*, Structure and function of tentacles, Reproduction, Stalk and lorica, Contractile vacuole, Nucleus and conjugation, Behaviour, Symbiotic bacteria, Phylogeny, Taxonomy of *Suctoria*, Methods of examination. Special Part: Suborder *Endogenea*, Family *Tokophryidae*, Genera: *Tokophrya*, *Erastophrya*, *Choanophrya*, *Rhyncheta*, *Digitophrya*, *Staurophrya*, *Trichophrya*, *Dendrosoma*, *Heliophrya*, *Endosphaera*, Family *Acinetidae*, Genera: *Acineta*, *Pseudogemma*, *Solenophrya*, *Loricophrya*, Suborder *Evaginogenea*, Family *Discophryidae*, Genera: *Discophrya*, *Rhynchophrya*, *Dactylostoma*, *Cyclophrya*, *Stylocometes*, *Dendrocometes*, *Trypanococcus*, Suborder *Exogenea*, Family *Podophryidae*, Genera: *Podophrya*, *Sphaerophrya*, *Gajewskajophrya*, *Parapodophrya*, *Manuelophrya*, *Mucophrya*, Family *Metacinetidae*, Genera: *Metacineta*, *Urnula*, Family *Spelaeophryidae*, Genus: *Spelaeophrya*, Dubious and incerte sedis species, References.

*Urceolariidae*

Introduction, General part, Morphology, Motility, Function of adhesive apparatus, Behaviour, Methods of species description. Special part. Genera: *Urceolaria*, *Trichodinella*, *Vauchomia*, *Trichodina*, *Semitrichodina*. Host-parasite list. References. Indexes: *Suctoria*, *Urceolariidae*.

In preparation:

J. Kołodziejczyk and A. Grębecki: Dynamics of the Submembrane Contractile System in Caffeine-derived Protoplasmic Droplets of *Physarum polycephalum* – E. V. Parfenova, S. Yu. Afon'kin, A. L. Yudin and R. N. Etingof: Characterization and Partial Purification of Mating Pheromone Excreted by Mating Type II Cells of the Ciliate *Dileptus anser* – I. Wita: Cytochemical Study of Dehydrogenase Activity in Two Euglenid Species of the Genus Parastasia Michajłow, 1966 – D. Chardez: Sur la multiplication de Centropyxis discoides et l'influence du milieu sur la morphologie de la theque (*Rhizopoda testacea*) – C. Kalavati and G. Krishna Murty: Morphology and Life-cycle of *Retractocephalus melanopli* sp. n. (*Didymophyidae, Eugregarinida*) from the Gut of *Melanoplus* sp. (*Orthoptera*) – J. J. Lipa and O. Triggiani: *Gregarina nymphaeae* sp. n., a New Eugregarine Parasite of *Galerucella nymphaeae* L. (*Coleoptera : Chrysomelidae*) – S. Ghose and D. P. Haldar: Role of Environmental Factors in the Incidence of Two New Species of Apicomplexan Parasites, *Hirmocystis triboli* sp. n. from Coleopteran Insects – I. B. Raikov and A. F. Volkonitin: A New Marine Psammobiotic Ciliate from the Japan Sea, *Trachelocerca obscura* sp. n. (*Ciliophora, Karyorelictida, Trachelocercidae*) – U. Buitkamp, W. Song und N. Wilbert: Ein neuer hypostomer Ciliat, *Pseudochlamydonella rheophila* sp. n. (*Pseudochlamydonellidae* fam. nov., *Pseudochlamydonella* gen. n.) im Aufwuchs eines Baches – S. Rakusa-Suszczewski and T. Nemoto: Ciliates Associations on the Body of Krill (*Euphausia superba* Dana) – S. Rakusa-Suszczewski and M. K. Zdanowski: Bacteria in Krill (*Euphausia superba* Dana) Stomach

Warunki prenumeraty

Prenumeratę **na kraj** przyjmują i informacji o cenach udzielają urzędy pocztowe i doręczyciele na wsi oraz Oddziały RSW „Prasa-Książka-Ruch" w miastach.

Prenumeratę ze zleceniem wysyłki **za granicę** przyjmuje RSW „Prasa-Książka-Ruch", Centrala Kolportażu Prasy i Wydawnictw, ul. Towarowa 28, 00-958 Warszawa, konto PKO BP XV Oddział w Warszawie Nr 1658-201045-139-11. Wysyłka za granicę pocztą zwykłą jest droższa od prenumeraty krajowej o 50% dla zleceniodawców indywidualnych i o 100% dla zlecających instytucji i zakładów pracy.

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