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A Study of the Genus *Spirodinium* Fiorentini. *Ciliata, Entodiniomorphida*

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Synopsis. The infraciliature of the genus *Spirodinium* was described. The differentiation of the adoral ciliary zone into the dorsal and ventral parts and the so called free cilia was showed. The structure of the cytopharynx was presented and the separation of the endoplasmatic sac from the ectoplast was characterized. A view on the systematic position of *Spirodinium* was expressed.

After the first description, given by Fiorentini (1980), and emendation by Hsiunga (1930, 1935), the genus *Spirodinium* was extensively elaborated in Strelkov's monograph (1939). Strelkov described its three known species occurring in the intestine of the horse, and two new species occurring in the zebra. Moreover, Strelkov (1939) excluded the genus *Spirodinium* from the family *Cycloposthiidae* Poche, 1913, comprising all the *Entodiniomorphida* that lived in the horse, and included it in the family *Ophryoscolecidae*. Davis (1941) described the species *S. equi* in detail. Recently, Imai et al. (1979) provided a description of the ciliature of the anterior part of the body of *S. equi*, using scanning microscopy. By light microscopy observations, Ike et al. (1983) described a new species *S. magnum* from the horse's intestine. The ciliature on the anterior part of genus *Spirodinium* body is differently described by early and recent authors, who, moreover, do not provide sufficient characteristics of the oral ciliature. The aim of the present paper was to give a more precise description of the ciliature of the anterior part of the body by applying silver impregnation and to investigate the oral and digestive apparatus.

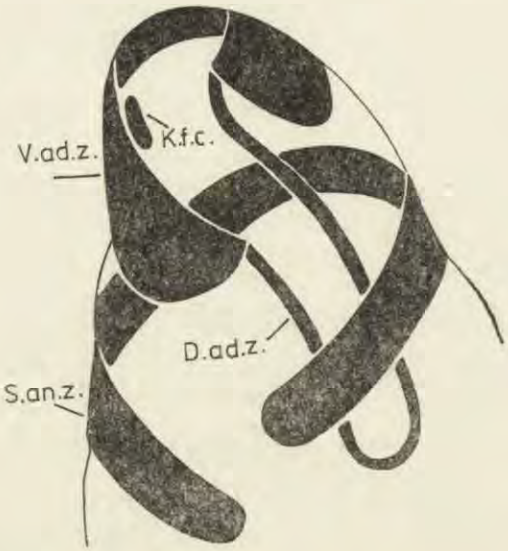

Fig. 1. Scheme of disposition of the ciliary zones in the anterior part of the body. Left-dorsal side view. The protozoan is represented as transparent. Ventral part of the adoral zone (V.ad.z.). Dorsal part of the adoral zone (D.ad.z.). Somatic anterior zone (S.an.z.). Kinetosomes of free cilia (K.f.c.)

the front of the body like a collar opening to the left dorsal side. The slightly widened ends of the band reach a shallow depression which constitutes the initial part of the vestibulum. The other part of the adoral zone, the dorsal one, has the form of a narrow ribbon. This ribbon penetrates the ciliate's body along the wall of the vestibulum. It forms a loop, running slightly to the dorsal side, and reaches far behind the level of the anterior somatic zone (Pl. I 2, 3, 5). A narrow dorsal ribbon starts at the end of the ventral part, which lies closer to the protozoan's dorsal side (Pl. I 2). Having come down to the bottom of the vestibulum, it turns round to the front and moves under the other end of the ventral part of the adoral zone (Pl. I 2, 6). At the posterior edge of the ventral part, on the protozoan's right ventral side, there can be seen a small group of kinetosomes of special cilia or free cilia (Pl. I 4, 5, 6). The ventral part of the adoral zone consists of rows of kinetosomes slightly slanting in relation to the long axis of the zone. The rows are parallel to one another, and, at the end closer to the body's ventral side, they are slightly divergent. Distances between the rows are very small, and the same in the whole area of the zone. In particular rows the kinetosomes lie close together. The dorsal part of the adoral zone consists of very short rows of 3–4 kinetosomes each. The rows are much more distant from one another than in the ventral part (Fig. 2 *A*). Cilia originating from this part of the adoral zone can be seen in the vestibulum (Pl. II 8). The group of free cilia consists of kinetosomes which get strongly impregnated with silver, are sparsely arranged, and form several rows (Fig. 2, Pl. I 4, 5).

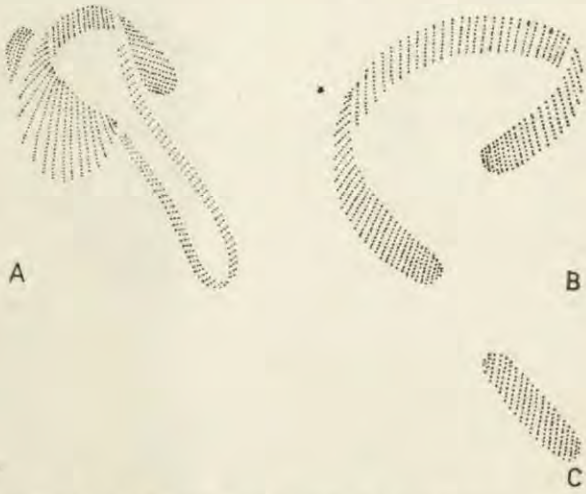

Fig. 2. Course of rows of kinetosomes in the ciliary zones, scheme. *A* — adoral zone. *B* — Somatic anterior zone. *C* — Somatic posterior zone

Such a structure of the adoral zone of the genus *Cycloposthium*, which is a type of the family *Cycloposthiidae*, was presented for the first time by Fernandez-Galiano (1959). This author also proved that the ventral part of the adoral zone develops from an anlage lying on the ventral side, while the dorsal one develops from a separate anlage lying on the dorsal side. Later on, such a structure of the adoral zone in *Tripalmaria dogieli*, *Tetratoxum unifasciculatum*, and, in a slightly altered form, in *Triadinium caudatum* was described by Wolska (1978 a, 1980 a, b, 1981).

In *Ophryoscolecidae*, the adoral zone is built according to the same general plan, but differs slightly from that of *Cycloposthiidae*. While in *Cycloposthiidae* the dorsal and ventral parts are differently developed and not united with each other, in *Ophryoscolecidae* the two parts, which develop from two separate anlages, unite to form a whole. Only in rare cases a gap may be seen between them. The rows of kinetosomes in the dorsal part have length and density similar to those in the ventral part. Moreover, in *Ophryoscolecidae*, the dorsal part does not turn round to the front after it has descended into the vestibulum. The above construction and development of the adoral zone in *Polyplastron multivesiculatum* was proved by Fernandez-Galiano (1958). Noirot-Timothee (1960) showed the same in other *Ophryoscolecidae*. Wolska (1965) added a description of free cilia in some species of *Ophryoscolecidae*.

In the genus *Spirodinium*, the somatic zones have a specific course, unknown in other *Entodiniomorphida*. The anterior zone encircles the

body spirally with at least one full turn and its ends are on the left side of the body (Fig. 1, Pl. I 2, 3, 6). The posterior zone is, as it was stated by Strelkov (1939), a prolongation of the anterior zone. In fact, it seems as if the anterior zone continued its course, after a break, in the posterior part of the body. The inclination of the ciliary rows in the posterior zone is the same as in the anterior one. In the anterior and in the posterior zones, both the density of kinetosomes in the rows and the density of the rows are very high (Fig. 2 *B*, *C*). Specimens which were silver impregnated indicate that the anterior somatic zone is completely separate from the adoral zone (Pl. I 6). The anterior part of the body is rich in protoplasmic depressions and elevations. The following of them are the most prominent ones: two lips of specific appearance, which border posteriorly the somatic zone (Pl. II 7–9, Pl. V 18), and a double lip bordering from the back the ventral part of the adoral zone. At its base, the lip splits into two leaves (Pl. II 8). One of them, which turns to the front, surrounds, like a bowl, the cilia of the adoral zone together with free cilia, and then turns itself towards the surface of the body, while the other hangs backwards down (Pl. II 7, 8). Also, the wall of the vestibulum is folded. Free cilia lie on a protrusion which is incised at its base, and cut with deep furrows (Pl. III 11, Pl. IV 14). The cilia grow in the furrows. In most of the cilia, their sections are not normal; they are either irregular polygons or elipses (Pl. III 12).

The cytostome which is equipped with "rideaux de microtubules" starts from the protozoan's anterior pole (Pl. III 11, Pl. IV 15) and courses along a deep vestibulum, surrounded with the loop of the adoral zone's dorsal part (Pl. V 18). The flattened passeway of the cytopharynx runs to the right side of the body and turns softly towards the back of the body. It is separated from ectoplast by a layer of microfilaments and microtubules; the latter group themselves into small nematodesmata (Pl. VI 24). This layer, the wall of the cytopharynx, forms folds penetrating the inside of the cytopharynx (Pl. VI 15, Pl. V 18). Bigger, rare nematodesmata surround it from the outside (Pl. IV 15). In a further part of its course, approximately at the level of the anterior part of the macronucleus, the cytopharynx's left wall becomes smooth, the folds disappear (Pl. II 10, Pl. III 13). Gradually, the cytopharynx widens and unites with an endoplasmatic sac or endoplast, which pushes out the ectoplast step by step in the posterior part of the body. (The justification of using the terms ectoplast and endoplast or endoplasmatic sac, instead of ectoplasm and endoplasm, is given by Strelkov (1939)). On the right, and partially dorsal side of the body the wall of the endoplasmatic sac is constructed in the same way as in the cytopharynx. The folds, which are broader and lower in this place, continue up till

the posterior end of the body. However, their number gradually decreases. At the level of the macronucleus' posterior part they are still numerous (Pl. II 10, Pl. III 13), at the posterior body end there are only a few of them: 4–6 (Pl. VI 23). In the remaining parts the boundary separating the endoplast from ectoplast (EE) is smooth (Pl. IV 16). As it gets closer to the cortex, it loses microtubules (Pl. IV 17), and its microfilaments finally fuse with the microfilaments of the cortex (Pl. V 20). It is only in a limited sector along the left body side that the wall of the endoplasmatic sac remains separate (Pl. VI 22), does not fuse with the cortex microfilaments and preserves its microtubules.

The structure of *Spirodinium's* cortex does not differ essentially from the structure of the cortex in *Triadinium, Tetratoxum* (Wolska 1978c, 1980 b) and *Cochliatoxum* (Senaud and Grain 1972), genera lacking skeletal plates similarly as *Spirodinium*. It should be stressed, however, that in *Spirodinium* the longitudinal microtubules are weekly developed and the circular microfilaments are strongly developed (Pl. V 21). Microfilaments unite into bundles (Pl. V 19), by which the continuity of the stratum is not broken; there only appear local condensations of microfilaments which surround the body. Also, branches penetrating the ectoplast are found from time to time, especially in the anterior part of the body (Pl. V. 21). In nonciliated parts of the body there occur, in the stratum of the cortex's microfilaments, scattered, barren kinetosomes (Pl. V 19). In ciliary zones, the dense substance, surrounding the bases of kinetosomes, fuses by longitudinal strands with the dense envelopes of neighbouring kinetosomes of the same row (Pl. VI 25), thus forming a common layer. I consider the fibers associated with kinetosomes to be kinetodesmal and transverse (Pl. VI 25).

Discussion

The ciliature of *Spirodinium* consists of three zones: the adoral zone, the anterior somatic zone and the posterior somatic zone. The adoral zone is divided into ventral part, dorsal part and free cilia. The dorsal part, composed of very short rows of cilia penetrates the deep vestibulum.

Imai et al. (1979) state, on the basis of photographs obtained from scanning microscopy, that the ciliature of the anterior part of *Spirodinium equi* consists of only one zone (adoral), which revolves two and one-half times round the vestibulum. Consequently, the number of zones on the body of *S. equi*, according to the authors, amounts to two, the adoral and the posterior somatic zone.

Wherefrom did this inconsistency in the assessment of the number and character of the ciliary zones in *Spirodinium* appear? Due to my lack of experience in scanning electron microscopy I can only make assumptions concerning this inconsistency. It may be that with the anterior part of the body so rich in folds and cilia as it happens to be in *Spirodinium*, the picture of the surface obtained from scanning microscopy is distorted. Perhaps the distance between the ventral part of the adoral zone and the anterior somatic zone, which is situated in close proximity to the former, became obliterated, thus making an impression of only one continuous zone of cilia. It is especially free cilia, which are hidden in furrows and constitute a small group, that might easily be masked by other cilia and cytoplasmatic lips. If, however, my presumption is not correct, i.e., if in the ciliate described by Imai et al. (1979) there was, in the anterior part of its body, only one zone of cilia, the adoral zone, it should then be recognized that we are dealing with a new genus, bearing in mind that in the diagnosis of the genus *Spirodinium* Fiorentini emend. Hsiung, there were mentioned three zones of cilia: the adoral zone, anterior somatic zone, and posterior somatic zone. Also, it seems to me that the ciliate recently described by Ike et al. (1983) as a new species, *Spirodinium magnum*, should be included in the presumed new genus. This species possesses, according to the authors' description, only two zones, one adoral zone in the anterior part of the body and one somatic zone in its posterior part. Of course, the adoral zone of this presumed new species remains to be investigated in detail with silver impregnation.

In the same manner as in *Spirodinium* the adoral zone of *Tetratoxum*, *Triadinium*, and, according to my last observations (unpublished), of *Ditoxum* and *Cochliatoxum* is built. The adoral zone of *Cycloposthium* (Fernandez-Galiano 1959) and *Tripalmaria* (Wolska 1978 a) is built similarly, except that in *Cycloposthium* and *Tripalmaria* the ventral part of the adoral zone lies on a retractable ciliophore, and may thus be drawn into the body. The genera *Ditoxum*, *Triadinium*, *Tetratoxum* (Strelkov 1939), *Cochliatoxum* (Strelkov 1939, Senaud and Grain 1972) and also *Spirodinium* do not possess this feature (Strelkov 1939).

The structure of the cortex and cytostome-cytopharyngeal complex of *Spirodinium* do not exhibit anything special as compared with *Triadinium* (Wolska 1979 c), *Tetratoxum* (Wolska 1980 a) and *Cochliatoxum* (Senaud and Grain 1972). In contrast, the bordering of endoplast by a fibrous layer differ from that in the above mentioned genera. In *Triadinium* and *Tetratoxum* the bordering is incomplete and appears as a weak layer of microfilaments occurring in the right side

of the body, which is where the nucleus lies; only this part of cytoplasm is separated from the digestive space. It seems that also in *Cochliatoxum* the microfilamentous layer constitutes a limitation of endoplast only in part of the cell, if we can judge from Senaud and Grain's statement (1972) on the subject of microfibrills in the cytopharyngeal region. It runs as follows: "Ces microfibrilles sont reliés a d'autres cordons de même nature qui entourent le macronoyau, certains régions endoplasmiques contenant de bactéries et à la strate souscorticale." The phrase: "certains régions endoplasmiques" suggests that the microfibrills separating endoplast from ectoplast do not occur everywhere in the cell. In *Spirodinium*, microtubules participate in separating the endoplasmatic sac from ectoplast or the cortex. Where they are absent, there occurs a large continuous layer of microfilaments. The bordering of endoplast by fibrous layer is complete, very distinct, and especially characteristic in the right, and partially dorsal, part of the body, by which it resembles *Tripalmaria* (Wolska 1978 b).

The place of the genus *Spirodinium* in the system of *Entodiniomorphida* used to change together with the changes of the system introduced by various authors. Strelkov (1939) excluded the family *Ditoxidae* from the old family *Cycloposthiidae*, and included in this new family the genera *Ditoxum*, *Triadinium*, *Cochliatoxum* and *Tetratoxum*, which have non-retractable ciliary zones and lack skeletal plates. On the other hand, he set *Spirodinium*, which also has non-retractable ciliary zones and lack skeletal plates, separately. Noting that certain features of this genus make it similar to ophryoscolecid ciliates, Strelkov (1939) created for this genus the subfamily *Spiriniinae* and included it in the family *Ophryoscolecidae*. Strelkov's view obtained no support from other authors; my present results of investigations concerning the adoral zone of *Spirodinium* do not support it, either. Noirot-Timothée (1960) excluded the subfamily *Spirodiniinae* Strelkov from *Ophryoscolecidae* and raised it to the rank of family (*Spirodiniidae*), equivalent to the families *Ophryoscolecidae*, *Polydiniidae*, *Cycloposthiidae*, and *Ditoxidae*, which belong to the order *Entodiniomorphida*. Latteur and Dufey (1967) performed a division of the family *Cycloposthiidae* Poche into two families: *Cycloposthiidae* and *Spirodiniidae*, taking for its basis the characteristics of the additional, i.e., somatic, ciliary zones. According to this division, the family *Cycloposthiidae* includes genera possessing several somatic ciliary zones in the form of ciliary bundles, and the family *Spirodiniidae* included genera possessing several somatic ciliary zones in the form of bands (franges au rubans ciliares). The family *Spirodiniidae* was divided into subfamilies. The subfamily *Spirodiniinae* included, according to the authors, *Spirodinium*, *Ditoxum*, *Tria-*

dinium, Cochliatoxum, Tetratoxum. Thus, the genus *Spirodinium* happened to occur in the same subfamily in which there had been these genera which Strelkov previously isolated as a separate family, *Ditoxidae.* In the new system of ciliates by Corliss (1979) the family *Spirodiniidae* includes, beside the genus *Spirodinium,* the genus *Cochliatoxum.*

In my opinion, the lack of skeletal plates and non-retractability of the adoral zone makes the genus *Spirodinium* similar to the genera *Ditoxum, Triadinium, Cochliatoxum* and *Tetratoxum.* However, the pronounced bordering of endoplast by a fibrous layer, and above all the general outline of its body and specific course of its somatic ciliary zones set *Spirodinium* apart from the above mentioned genera. Besides, the two last features distinguish *Spirodinium* from the other *Entodiniomorphida.* I consider it proper, according to Noirot-Timothée's conception (1960), to establish for the genus *Spirodinium* Fiorentini, and only for this genus, an independent family *Spirodiniidae* within the order *Entodiniomorphida.*

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EXPLANATION OF PLATES I–VI

1 — Hematoxyline staining; 2–6 — silver impregnation, 7–10 — semithin sections, 11–25 — ultrathin sections

1: *S. equi.* Right side general view. Adoral zone of cilia (Ad.z.). Somatic anterior zone (S.an.z.). Somatic posterior zone (S.p.z.) 1000 ×

2: *S. confusum.* Left side view. Disposition of the ciliary zones in the anterior part of the body can be seen. 1000 ×

3: The same specimen. Focus on the right side. 1000 ×

4: *S. equi.* A part of the adoral zone is seen from the left side. Group of kinetosomes of special cilia or free cilia (K.f.c.). 1500 ×

5: *S. confusum.* Right side view. Ventral part of the adoral zone. (V.ad.z.). Dorsal part of the adoral zone (D.ad.z.). Somatic anterior zone (S.an.z.). Kinetosomes of free cilia (K.f.c.). 1500 ×

6: Ventral side view. Dorsal narrow band of the adoral zone pushes under the ventral part of the adoral zone. Kinetosomes of free cilia near by. Somatic anterior zone (S.an.z.). 1700 ×

7: *S. uncinucleatum.* Longitudinal slightly oblique section near the right body side. Lip of the adoral zone (L.ad.z.). Somatic anterior zone (S.an.z.). Posterior contractile vacuole (C.v.p.). Vestibulum (arrow). 1500 ×

8: Longitudinal slightly oblique section. Cilia of the adoral zone (Ad.z.). Lip of the adoral zone (L.ad.z.) splitting into two parts. Lip of the somatic anterior zone (L.s.an.z.). Cilia into vestibulum (arrow). 1500 ×

9: Longitudinal oblique section near the right body side. Somatic anterior zone and their lip is visible. Contractile vacuole is situated posteriorly to the somatic zone. 1500 ×

10: Oblique section near the middle of the body. Right side (R). Left side (L). 1500 ×

11: *S. confusum.* Oblique section of the anterior body part at the level of the tubercle with the free cilia (arrow). Cytostome and cytopharynx are visible. 8800 ×

12. Transverse section of the free cilia. Some of them are deformed. 17 600 ×

13: Transverse section at the level of the anterior part of the macronucleus. The right wall of the cytopharynx is folded. 6200 ×

14: *S. equi.* Section of the tubercle with free cilia. 16 800 ×

15: *S. confusum.* Oblique section of the anterior body part at the level similar to that in photo. 11. Cytostome and cytopharynx are visible. Vestibulum (V). 17 000 ×

16: Transverse section, slightly oblique. Longitudinal bars of dense substance (L.b.). Microfilaments of the cortex (arrow). Ectoplast/endoplast boundary (EE) consisting of microfilaments and microtubules. 24 000 ×

17: Oblique section. Ectoplast/endoplast boundary devoid of microtubules (arrow). 23 000 ×

18: Longitudinal oblique section at the level of the somatic anterior zone (S.an.z.). Cytostome and cytopharynx are visible (arrow). Vestibulum (V). 6200 ×

19: Oblique section. Microfilaments in bundles. Kinetosome (arrow). 17 600 ×

20. Oblique section. Longitudinal bars of dense substance (L.b.). Transverse microfilaments (Mf.) Ectoplast/endoplast boundary (EE). 24 000 ×

21: *S. equi.* Section nearly perpendicular to the surface in the anterior part of the body. Microtubules (T). Longitudinal bars of the dense substance (L.b.). The layer of the microfilaments (M.f.). 21 000 ×

22: Transverse section of the posterior body part. Left side. Ectoplast/endoplast boundary (EE) not fused with microfibrils (Mf.) of the cortex. 24 000 ×

23: The same cutting. Right side. In the folded ecto/endoplast boundary microtubules in groups of 5 or 6 (arrow). 24 000 ×

24: Fragment of a section of the cytopharynx in the initial part. Groups of microtubules — small nematodesmata (arrow). 24 000 ×

25: Section of the somatic posterior zone of cilia. The dense substance underlying kinetosomes and longitudinal strands (arrows). Kinetodesmata (Kd.), transverse microtubules (Tr.). 23 000 ×

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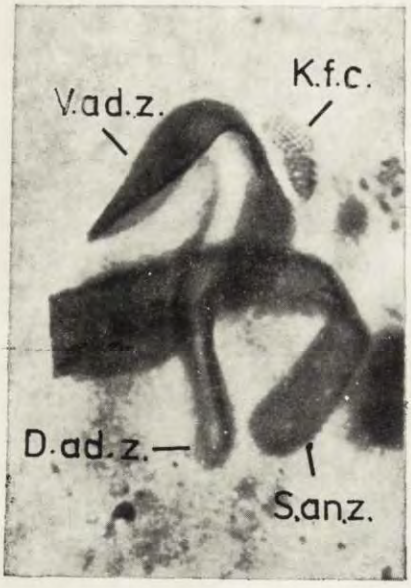

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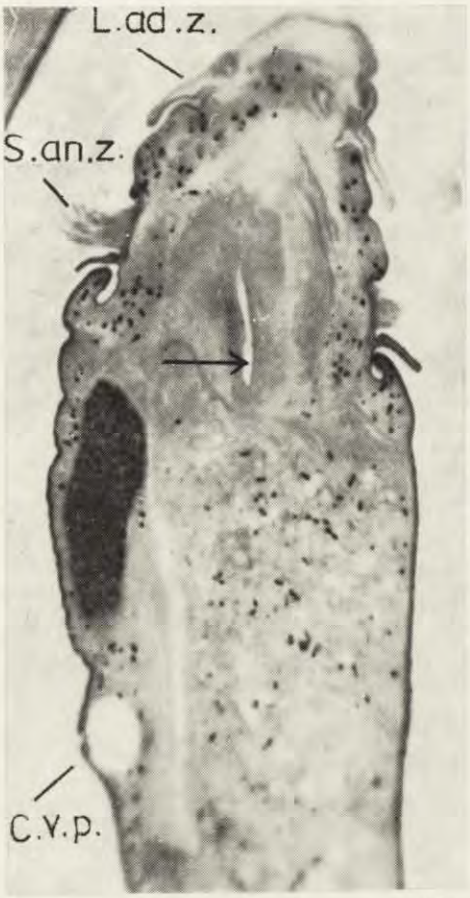

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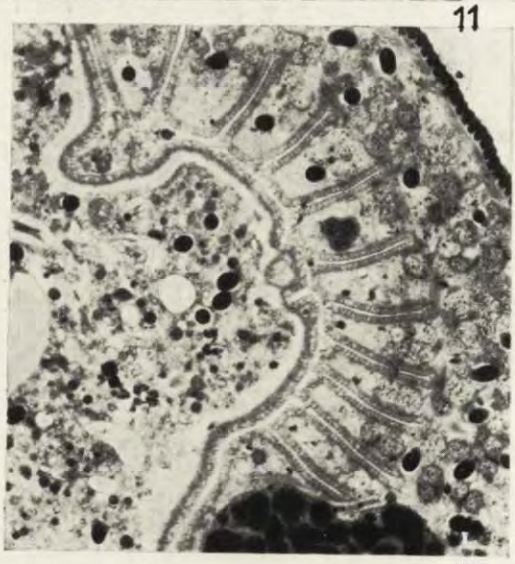

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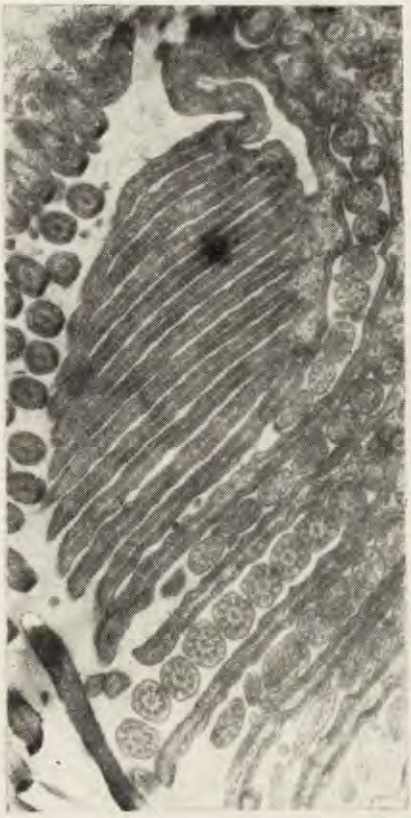

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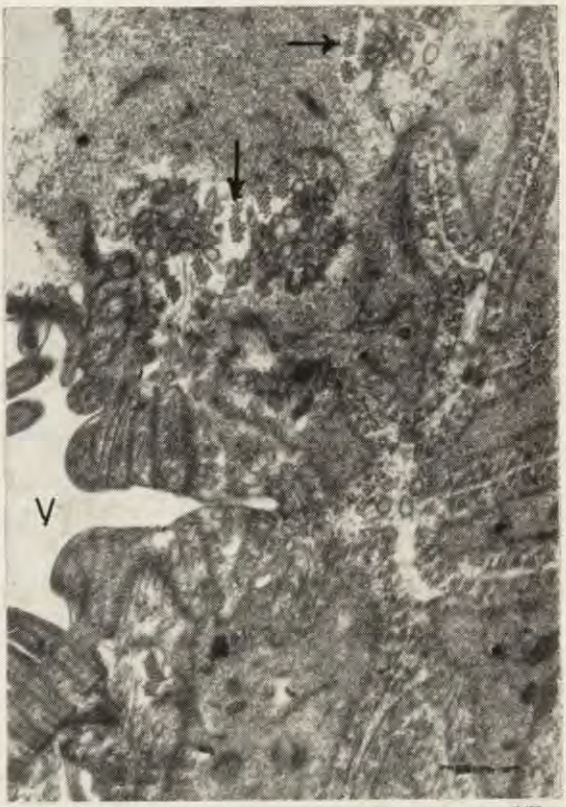

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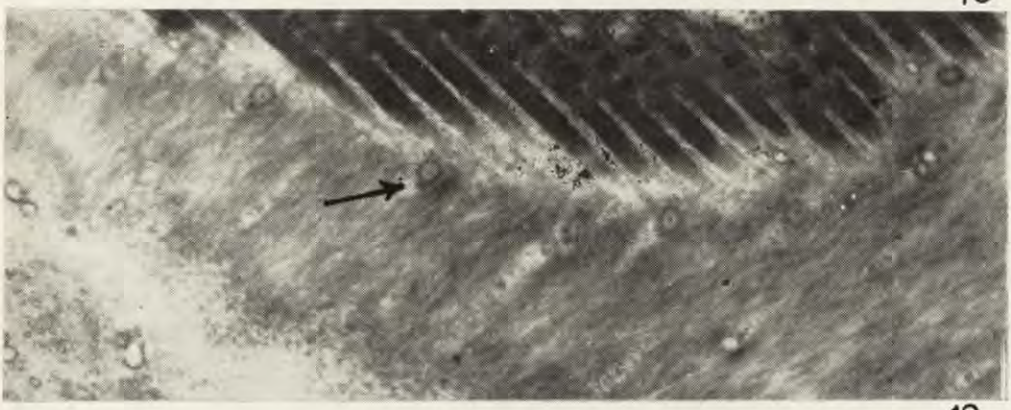

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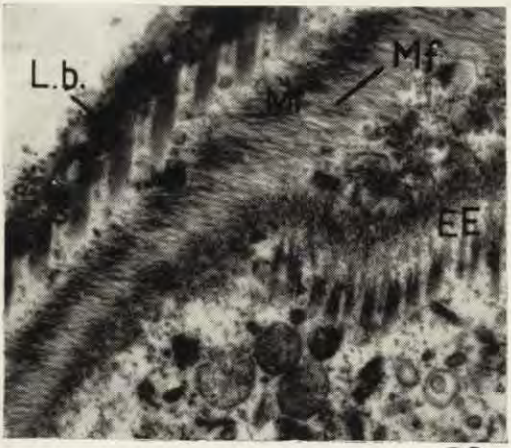

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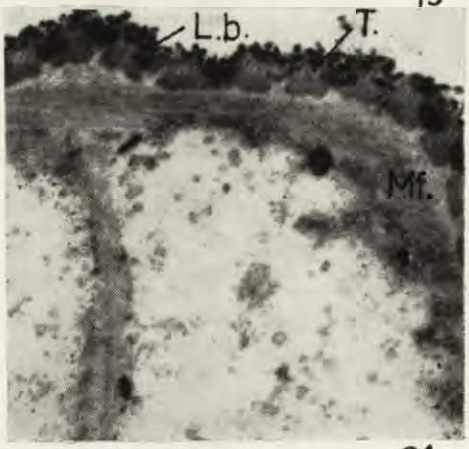

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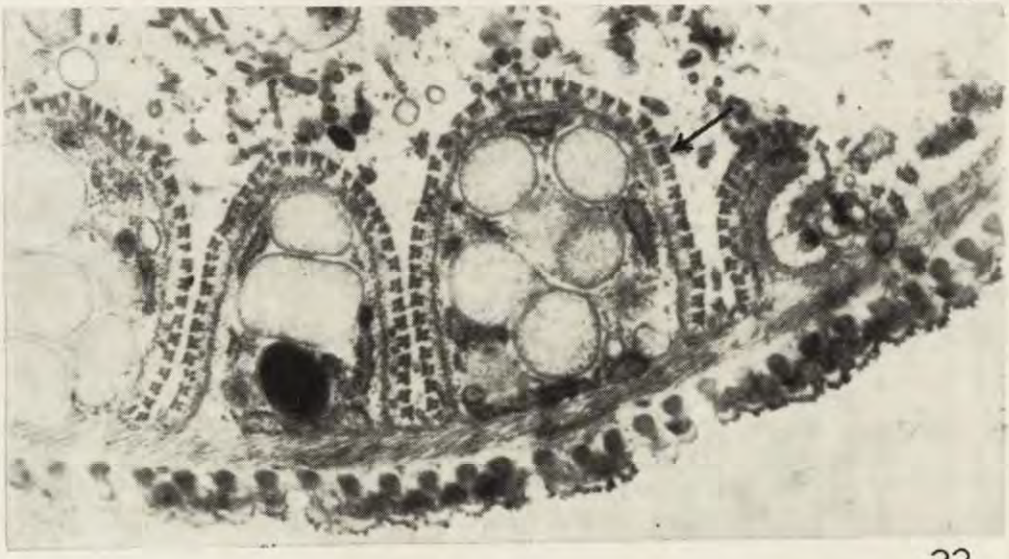

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Diversity and Ecology of Psammolittoral Ciliates (Protists) from Shediac Harbour, (N.B.), Canada

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Synopsis. This is the first study devoted specially to psammolittoral ciliates from eastern Canada, which incidentally also happens to be the first from this country (as revealed by telereference search). Two locations were chosen, one closer to the discharge of the Scoudouc river in the Shediac harbour and the other farther away towards the open sea. A study of 14 sand samples from each location collected during a period of three months has brought to light the existence of some 14 species belonging to ciliates besides some foraminifers, nematodes, copepods and a rich bacterial and diatom flora. The two locations are considered to be fairly rich in food supply for the ciliates in the form of bacteria, diatoms, decomposed organic particles and presumably have an insignificant number of predators. The above type of biotic ecology permitted a fairly stable population of ciliates during the summer of 1982.

The occurrence of protists in the interstitial spaces of sand banks of estuaries and beaches of the oceans has been demonstrated by the sporadic works of Sauerbrey (1928), Kahl (1923, 1930, 1933, 1935), Chatton and Lwoff (1949), and Fauré-Fremiet (1950, 1951, 1954). A renewed enthusiasm in the study of psammolittoral ciliates is evidenced by a large number of publications from various parts of the world during the later half of this century. This is witnessed by a series of notable studies published from other parts of the world by French (Dragesco, 1953, 1960, 1963 a, b,. 1966 a, b, Grolière and Detcheva 1979), Russian (Agamaliev 1967 a, b, 1968–1975, Burkovsky 1969, 1970 a, b, c, 1971, Raikov 1960, 1962, 1963, Raikov and Kovaleva (1968), English (Gray and Ventilla 1973, Barnes

et al. 1976, Gray 1976, Hartwig and Parker 1977, Parker 1976, 1978, 1979), Danish (Fenchel 1968, 1972, 1973, 1978), Bulgarian (Detcheva 1981, Golemansky 1981), Swedish (Ankar 1979), Indian (Rao and Ganapati 1968, Rao 1969, 1974), Japanese (Sudzuki 1979) and American (Todd and Low 1981) protozoologists.

From United States of America noteworthy contributions on the study of psammolittoral ciliates have been made by Fauré-Fremiet (1951) from Cape Cod, Borror (1963 a, b, 1965, 1968, 1972, 1973) from the Gulf of Mexico and Florida coasts, and Jones (1974) from Mobile Bay of Alabama. From South America studies by Kattar (1970, 1976) on Brazilian coast and from Bermuda the contributions by Hartwig (1977, 1980) are noteworthy. The genera and species reported in the world-wide studies indicate many psammolittoral ciliates to be cosmopolitan in distribution.

Three telereference searches by the author have indicated a total absence of any published study on the sand dwelling ciliates from Canada. Thus the present study will be the first report of the occurrence and diversity of the psammolittoral ciliates from Eastern Canada.

Most sand beaches are often inhabited with varying densities and diversities of living and dead macro and micro flora and fauna specially up to the high tide zone. Occasionally, the clean sandy stretches seem to be completely barren and devoid of life forms but a microscopic examination of such sands, with proper recovery techniques, has in many cases revealed the presence of microscopic bacteria, algae (mostly diatoms, dinoflagellates, occasionally fungal spores), nematodes and protists, etc. Ecologically and physiologically, the flora and fauna of such a habitat must be able to adapt and to endure successfully the fundamental requirements of survival and propagation of their progeny. These microfloral and microfaunal communities are exposed to varying periods of freezing temperatures (in cold countries) and periods of dessication (intertidal intervals) and innundation. Only a few studies have been done previously with a view to investigate the ecology, occurrence and diversity of estuarine and typically psammolittoral fauna. Some of the earlier work done by Kahl (1933, 1935) and Fauré-Fremiet (1950, 1951) from the beaches of Europe and United States respectively have indicated the occurrence of an interesting and moderately rich protozoan fauna dwelling in the interspaces of different size grades of the sand grains. The present study was initiated in 1982 to investigate if and which type of ciliates were present in the nearby Shediac estuary and the beach of the Shediac region located in the eastern part of the Province of New Brunswick.

Area of Study

Shediac harbour receives fresh water from Scoudouc river and Shediac river which flow into it from south and west respectively, while on the north eastern side it communicates directly with the oceanic waters of the Northumberland Strait. The area of study is situated on the land mass comprising the south-east of the Shediac harbour. The topography of this land mass consists of a combination of some rugged stretches jutting into the harbour and some areas exposing coarse to fine sand beaches. Two stations were chosen as shown in the Fig. 1. Two considerations were taken into account for the choice of these stations, the first being that Station 1 should represent an estuarine location and Station 2 a more marine environment. The second consideration being to select the locations least frequented by the visitors and local residents to ensure the more natural conditions. The two sites are about 2.5 to 3 km apart.

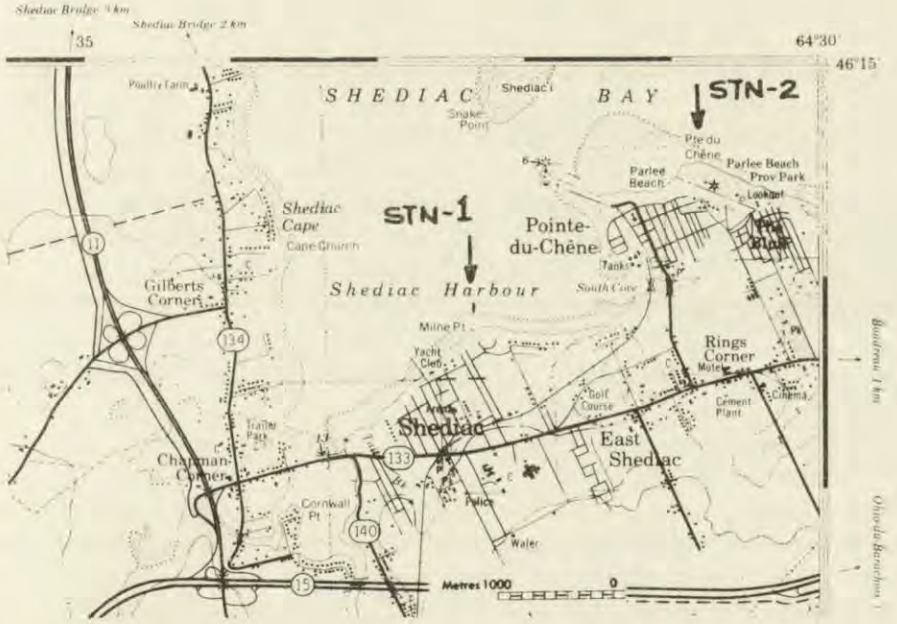

Fig. 1. The area of study

Method of Study

In order to ensure a greater consistency of the diversity of population and the numerical representation of the ciliates during the period

of observation, the sampling was done once a week regularly for a three month period (end of May to end of August).

The sampling sites at the two stations were located about 4 m from the low tide level. Every week samples of wet sand were collected from the two sites from depths between 0–10 cm with the help of a hollow metal pipe 30 cm long and ca 5 cm in diameter with markings on the outside to indicate the depth penetrated in the sand. To collect the samples, the tube was vertically pushed in the sand to marked depth of 10 cm and then the sand and silt around the pipe was scooped out to reach the bottom of the pipe. The bottom of the tube was closed with one hand and the tube lifted by the other hand with sand sample. The sample was emptied in a bottle containing the tidal water from the same locality, labelled and transported to the laboratory. The temperature (of the wet sand at the time of collection), pH and salinity were determined for each sample collected.

To observe the diversity of protists, each sample collected was transferred to large sized finger bowls along with its respective estuarine or saline water in such a way that the sediment was fully submerged under water to a height of about 1 cm. The finger bowls were covered with "parafilm" (to permit exchange of air between the finger bowls and the atmosphere and to protect the finger bowls from atmospheric contamination) and left overnight at room temperature.

The samples were examined the next day in two fractions: the supernatant water was drained out in a separate bottle and centrifuged at 2500 rpm to concentrate the sediment and the micro-organisms still in water. After draining out this water, the sediment was examined in watch glasses and on slides with a few drops of water from the decanted fraction to which was added a few drops of Methyl cellulose to slow down the movement of ciliates.

The other fraction was examined by taking out a small amount of sand in a petri-dish to which was added a small quantity of decanted water obtained from fraction 1. To this were added a few drops of 12% $MgCl_2$ solution which narcotizes small metazoans, reducing the thigmotactic adherence of ciliates to sand particles and releases them for better observation under the low power binocular microscope (Fauré-Fremiet 1951). The sand was pushed to one side by tilting the petridish and shaking gently. The protists thus released were observed under a binocular microscope. The gliding and swimming psammophilous ciliates were picked up by a capillary pipette and transferred to (grooved) slides for a more careful observation and identification under the high power microscope. The excess water from the grooved slides

was removed by a simple technique of absorbing the water by touching the water film by a corner of the blotting paper, while constantly observing the ciliate through the microscope until just enough water was left for the ciliates to make normal gliding or swimming movements. The detailed examination for final identification was done under high-power microscope.

The granulometric characteristic of the mean size range of the sand from the two locations (stations 1 and 2) was determined under a petrological microscope by a micrometric estimation. The grain sizes at both locations varied from 0.05 to 1.5 mm and did not show a spectacular difference except for the fact that station 1 had a larger percentage of coarse grains and was richer in organic particles as compared to station 2 which had a larger percentage of finer sand-silt with moderate amounts (5 to 10%) of micaceous flakes.

Observations

During the period of study, May 28, 1982 to August 27, 1982, a total of 14 samples from each station were collected. The salinity variations at the two station ranged from 12.3% to 28% and 22% to 31.5% respectively. Decreased salinities were observed after heavy and continuous rainy spells. The temperatures recorded at the two stations during the day varied between 14 to 26.5°C and pH ranged between 7.4 to 8.1. Table 1 presented below indicates the distribution of the ciliates identified from the two stations.

Table 1

	STN 1*	STN 2**
Aspidisca sp.	×	–
Blepharisma clarissimum var. *arenicola* Kahl	×	×
Centrophorella lanceolata Fauré-Fremiet	×	×
Coleps similis Kahl	×	–
Coleps spiralis Noland	–	×
Euplotes affinis Dujardin	×	–
Euplotes spp.	×	×
Geleia simplex Fauré-Fremiet	–	×
Lacrymaria lagenula Clap. et Lach.	×	–
Litonotus fasciola (Ehr.) Kahl	×	×
Mesodinium sp.	–	×
Placus socialis (Fabre-Domergue) Noland	×	×
Remanella obtusa Fauré-Fremiet	–	×
Spirostomum teres Clap. et Lach.	×	×
Strombidium cinctum Kahl	×	×
Strombidium cf. *pelagicum* Fauré-Fremiet	–	×
Uronema marinum Dujardin	–	×

* Milne Point.
** Point du Chene — Parlee Beach.

General Remarks

The present study of the psammolittoral ciliate fauna from Shediac harbour clearly indicates the occurrence of a moderately rich and diverse population, which means that the microecological conditions for the support of the ciliate fauna should be rated as fair to good. A recent study by Lakshminarayana and Jean-Pierre (1975) carried out in the western part of Shediac harbour indicated "the presence of more than 1000 organisms/100 ml coliform population". This type of pollution has been interpreted by the authors as sewage pollution. Their study did not include the south eastern part of the Shediac harbour, hence no precise information is available for the locations.

At stations 1 and 2, the sands and silts present in the reaches of the tidal water are innundated twice every 24 h period. Thus from an ecological point of view, these ciliates occurring in the interstitial spaces must possess the capacity to resist a certain degree of water deprivation and dessication during the day, specially on hot sunny days when the interstitial spaces of the upper few centimeters of sand becomes devoid of water. At both the localities investigated, it was observed that on hot sunny summer days, the upper 5–10 mm of the sand usually became completely dehydrated in about 2 to 3 h time. These sand samples from the upper layers rarely showed the presence of any ciliates and those noticed were dead. However, on rainy days, these layers showed a better yield of the ciliates. This observation leads to a logical presumption that the ciliates move downwards as the water and sand interface moves down. The overall ecological factors which influence the ciliate populations of beaches have been discussed by Dragesco (1960) and Fenchel (1969). An abundance fluctuation study of mesoporal organisms done by Ankar (1979) in the upper 5 cm of sediments indicated that the abundance of these organisms depended on the position of black sulfide layer. Sands from both areas of study were also examined under petrological microscope for their detritus contents. A rough estimate revealed that the sands from station 1 were having about two times more organic particles in the form of decaying plant and animal matter than those of station 2. The latter, however, had a more profuse bacterial and diatom population. Most of the ciliates recovered from the two localities feed on bacteria and algae, except *Litonotus* which is reported to be carnivorous. A rich diatom flora was noticed to be present in the interstitial spaces. From a pH and salinity tolerance point of view, several of the ciliates commonly reported from fresh waters seems to have evolved tolerant strains and thus were observed in estuarine and marine sands. During the three month period, from May 28 to August 27, no

dramatic change in the total population of the ciliates was noticed at any of the two stations. However, the diversity in genera and species of ciliates increased significantly, along with a moderate increase in the total number of diatoms and nematodes, during the months of July and August. This rise in number and diversity of the ciliate population may be due to a large number of physico-chemical and biological environmental factors (Stout 1955, 1956, Mann 1972), which influence the excystement, rate of reproduction, supply of food, decrease in the number of predators, etc.

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Adaptation of Stomatogenesis and Cell Division in *Tetrahymena pyriformis* GL to the Continuous Presence of Colistin in the Medium

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Synopis. The effects of non-lethal concentrations of colistin in the medium upon oral development and cell cycle in *Tetrahymena pyriformis* GL were investigated. The disturbances in the process of stomatogenesis were found to consist of a temporary blockage in the formation of the oral apparatus and the resorption of the oral primordium. The rate of recovery depended on the antibiotic level in the culture. In *Tetrahymena pyriformis* resorption of the oral primordium effected by colistin is characteristically an all-or-none process. The regulation of the first cell cycle in the ciliate brought about by the drug before the mechanisms responsible for the physiological adaptation are triggered involves the phenomenon of set-back in the cell cycle. The stabilization point in the cell cycle was also observed with respect to colistin. It occurs not later than 45 min prior to the completion of cytokinesis, i.e., between stage 4 and stage 5 of the oral development.

The manner in which the cell cycle is regulated in the continuous presence of cell metabolism inhibitors constitutes an important problem of contemporary biology. The cell cycle consists of a sequence of morphogenetic events leading to the reconstruction of the entire ensemble of cell organelles in both daughter cells.

In *Tetrahymena* the cell cycle includes a distinct stage of cortical morphogenesis particularly susceptible to agents blocking protein synthesis (Frankel 1965, 1967 b, Frankel and Williams 1973). Disturbances in the division morphogenesis lead to the lengthening of cell cycle and disorders of oral apparatus.

Formation of a new oral apparatus, or stomatogenesis, is a characteristic stage of cell cycle in ciliates. It has been established, however,

that stomatogenesis is not a prerequisite for cell division in some ciliates (Frankel 1961, Nanney 1967). Stomatogenesis in *Tetrahymena* has been found to consist of six stages (Frankel 1961). Studies with cycloheximide have demonstrated that the so-called stabilization point occurs early in stage 5 (Frankel 1962). After that cells exposed to a given physical or chemical agent divide and the development of the oral apparatus is normal. On the other hand the action of the agent prior to stabilization point induces a blockage of cell division as well as a partial or complete set-back in the formation of oral apparatus, or resorption (Frankel 1962). According to the agents employed and their concentration or intensity, the rate of resorption varies while the stabilization point may occur at another stage (Mitchison 1978). After the inhibiting effect of the drug has been reversed, e.g., due to physiological adaptation, *Tetrahymena* complete the previously arrested process of cytokinesis and morphogenesis. Accordingly, the study of cortical morphogenesis in the presence of a cell metabolism inhibitor would at the same time allow to establish the nature of cell cycle regulation in *Tetrahymena* induced by the inhibitor.

Material and Methods

Material and Conditions of Cell Culture

The organism used in the study was an amicronucleate strain of the ciliate *Tetrahymena pyriformis* GL.

Stock cultures were maintained axenically in 20 ml test tubes (16 mm in diameter) containing 5 ml of medium (1.5% proteose peptone + 0.1% yeast extract: Difco). The water used was three times distilled and deionized. To ensure optimum thermal conditions the cultures were placed in an incubator at 28°C. The medium was weakly acidic at a pH of 6.5. The stock cultures were inoculated every seven days by transferring 0.5 ml of cell suspension to 5 ml of the medium. The experimental cultures, i.e., with colistin or without the antibiotic (control) were maintained in 200 ml flasks containing 50 ml of the medium, its composition and thermal conditions being the same as in stocks. The exponential growth phase was achieved by a triple inoculation of stock cultures every 24 h. The experiments were started 3 h after the last inoculation. The ciliates used in the study divided asynchronously.

Antibiotic Employed

The antibiotic employed, which is a mixture of colistin A (= polymyxin E_1) and colistin B (= polymyxin E_2) was produced by the Polfa Pharmaceutical Works at Tarchomin. In the present study colistin was used under the form of sulphate salt. As a therapeutic agent the antibiotic is fairly stable at a pH of 2 to 6 (Ko-

rzybski et al. 1977). On the grounds of initial exploratory trials in the presented conditions and of earlier findings (Szablewski 1981), two non-lethal concentrations of the antibiotic were selected, 0.05 mM/l = 0.07 g/l and 1 mM/l = = 1.4 g/l.

Colistin is a polypeptide antibiotic acting upon the cell membrane though not penetrating inside the cell (Few and Schulman 1953, Newton 1953, 1954a, b, 1955, Sebek 1967, McKay and Kay 1964, Axline et al. 1967, Jawetz 1970). By getting built up in the bacterial cell membrane (Kuryłowicz 1979), colistin changes its structure and enhances its permeability (Sebek 1967, Russel 1977), thus acting upon bacteria in much the same way as detergents (Korzybski et al. 1977). As a result such compounds as purines, nucleotides, simple sugars and aminoacids are released from the cell (Russel 1977).

Statistical Methods

The findings were compared by variance analysis "Two way ANOVA with replication" (Sokal and Rohlf 1969) at the confidence level of 95%.

Experimental Procedures

Due to the nature of the study, the experiments were conducted on exponentially growing populations. Particular phases of *Tetrahymena* cultural growth were determined according to the criteria of bacterial cultural growth curves by Monod (Satir 1967).

(A) A study of multiplication rate of *Tetrahymena pyriformis* GL

Three hours after inoculation proper concentrations of the drug were added to experimental cultures. The moment when the antibiotic was added was referred to as zero time. The culture density, measured by means of an electronic cell counter, was then ca. 2500 cells/ml. An increase of cell number in time (dV) in particular cultures was calculated according to the formula

$$dV = \frac{V_t}{V_0},$$

where V_t — culture density at a given time point, V_0 — culture density at zero time.

(B) The course of stomatogenesis

The antibiotic was added at the same time as in experiment A. The samples were taken at 0, 0.75 (45 min), 2, 3, 4.5, 6, 9, 12, 15, 18, 21 and 24 h, following a partially modified system of Frankel (1969). Particular stages in the formation of a new oral apparatus were determined according to Frankel (1967a). Specimens for the observation of the developing oral structures were prepared using a Chatton-Lwoff procedure modified by Frankel and Heckmann (1968). The investigated sample comprised ca. 200 cells. The observations were performed using a light microscope with immersion, the total magnification being 1600 ×.

Results

(A) The effect of colistin on the multiplication rate of ciliates

Colistin in the culture medium was found to affect the *Tetrahymena* multiplication rate. At 0.05 mM/l colistin produced the lengthening of the lag phase by ca. 20 min as compared to controls. In later stages the rate of multiplication in the investigated sample was similar to that in the control culture. A variance analysis established that the differences between the two samples in the dynamics of cell multiplication over the 12 h duration of the experiment were not statistically significant. Since the assumption was that further changes in the cell number would follow the same pattern as in the control sample, observations of the cultural growth phases with colistin at 0.05 mM/l were stopped at that time (Fig. 1). Additionally, a connexion between changes in the culture den-

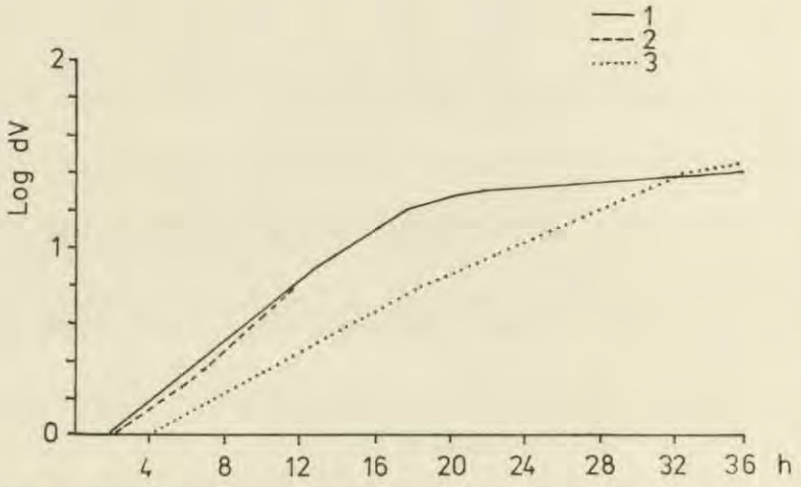

Fig. 1. The multiplication rate of *Tetrahymena pyriformis* GL according to colistin concentration in the medium. The culture treated with 0.05 mM/l colistin was under observation for 12 h. *1* — control, *2* — colistin 0.05 mM/l, *3* — colistin 1 mM/l

sity and the duration of the experiment was established, i.e., increase of cell number was found to be proportional to the duration of their exposure to colistin. The correlation was statistically significant.

An addition of 1 mM/l colistin to the culture produced more marked changes in the course of cultural growth phases. The lag phase lasted throughout the first four hours of the experiment, i.e., ca. 2.5 h longer than in the control. The course of other cultural growth phases in the discussed sample also differed from the control culture (Fig. 1). The

variance analysis demonstrated that at 1 mM/l colistin induced statistically significant changes in the rate of *Tetrahymena* multiplication as compared to the other two samples. In the sample containing 1 mM/l colistin the increase of cell number in time was statistically significant.

(B) The effect of colistin upon the course of stomatogenesis

The study demonstrated that there is a connection between the course of stomatogenesis in *Tetrahymena* and the antibiotic level in the culture as well as the growth phase of the culture.

In the control sample the fraction of cells with the developing oral primordium was at its lowest throughout the lag phase (8.5%), i.e., between 0 and 2 h. The value subsequently increased to 12% at the beginning of the exponential growth phase. In the control sample the maximum number of cells undergoing stomatogenesis was found in exponentially growing populations. The fraction of cells with the oral primordium averaged then 17% remaining at that level until the completion of the experiment (Table 1, Fig. 2).

At 0.05 mM/l colistin arrested stomatogenesis after 45 min of the ciliates exposure to the antibiotic. After two hours there appeared organisms undergoing particular stages of stomatogenesis (5.5%). The next sample collected 3 h after 0 time showed the course of oral development identical to the one observed in the control. Also after 4.5 h of the experiment colistin at 0.05 mM/l was not found to affect the process of stomatogenesis. The same was observed until the end of the experiment and the difference in the number of cells with a developing oral primordium between the investigated population and the control reaching 2% cannot be considered statistically significant (Table 1, Fig. 2).

After 2 h of *Tetrahymena* exposure to colistin at 1 mM/l no organisms with a developing oral primordium were observed. Three hours after 0 time there appeared a fraction of cells (1%) undergoing various stages of stomatogenesis. Subsequently the number of organisms with a developing oral primordium gradually increased. In the investigated sample the number achieved the control level after 12 h. The variance analysis demonstrated that differences in the course of stomatogenesis due to the addition to the culture of 1 mM/l colistin were statistically significant as compared to the course of the process in the other samples (Table 1, Fig. 2).

However, no effect was established of the investigated colistin concentrations upon the frequency with which particular stages of oral development occurred in *Tetrahymena* (Fig. 3). The findings do not reveal statistically significant differences.

The presence of colistin in the culture produced also other disorders in the course of oral development in the investigated ciliates. Unlike

Table 1

The number of cells undergoing particular stages of stomatogenesis according to colistin concentration and the duration of the experiment

Time n hours		Stage of Stomatogenesis							R
		0	1	2	3	4	5	6	
0	Control	181	2	4	4	2	1	6	0
	Colistin 0.05 mM/l	183	6	2	3	0	0	6	0
	Colistin 1 mM/l	181	6	3	2	1	0	7	0
0.75	Control	183	1	4	2	1	1	8	0
	Colistin 0.05 mM/l	190	0	0	0	0	0	0	10
	Colistin 1 mM/l	191	0	0	0	0	0	0	9
2	Control	180	3	3	5	1	0	8	0
	Colistin 0.05 mM/l	182	0	0	2	3	5	1	7
	Colistin 1 mM/l	187	0	0	0	0	0	0	13
3	Control	176	4	5	3	1	1	10	0
	Colistin 0.05 mM/l	175	0	3	4	4	4	10	0
	Colistin 1 mM/l	188	0	0	0	1	1	0	10
4.5	Control	175	5	6	2	0	2	10	0
	Colistin 0.05 mM/l	176	3	2	2	3	3	11	0
	Colistin 1 mM/l	184	1	1	2	2	3	2	5
6	Control	165	6	7	5	4	3	10	0
	Colistin 0.05 mM/l	167	5	5	4	2	5	12	0
	Colistin 1 mM/l	178	0	2	5	3	4	6	2
9	Control	166	3	7	3	8	1	12	0
	Colistin 0.05 mM/l	162	6	3	5	3	8	13	0
	Colistin 1 mM/l	171	4	2	3	6	5	8	1
12	Control	166	4	4	4	5	5	12	0
	Colistin 0.05 mM/l	162	4	4	5	5	7	13	0
	Colistin 1 mM/l	164	4	3	7	7	4	11	0
15	Control	166	4	3	4	6	6	11	0
	Colistin 1 mM/l	162	6	4	4	6	7	11	0
18	Control	166	4	4	4	5	6	11	0
	Colistin 1 mM/l	165	4	4	5	4	7	11	0
21	Control	168	4	3	5	5	5	10	0
	Colistin 1 mM/l	166	5	5	5	4	5	10	0
24	Control	168	3	5	6	4	3	11	0
	Colistin 1 mM/l	167	3	5	4	5	4	12	0

R — the number of cells suffering resorption of the oral primordium, N — 200 cells.

controls, some of the treated cells suffered resorption of their oral primordia.

After 45 min of treatment with colistin at 0.05 mM/l, resorption of the oral primordium occurred in 5% of the organisms. After 2 h the number decreased to 3.5%, while after 3 h and later the phenomenon was not observed.

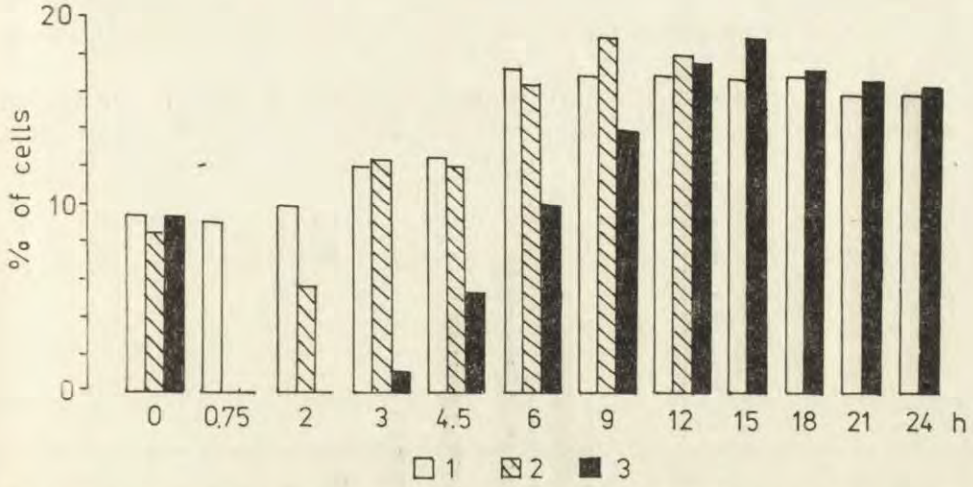

Fig. 2. The percentage of cells undergoing oral development according to colistin concentration in the medium and the duration of the experiment. The medium containing 0.05 mM/l of the drug was under observation for 12 h. N — 200 cells, *1* — control, *2* — colistin 0.05 mM/l, *3* — colistin 1 mM/l

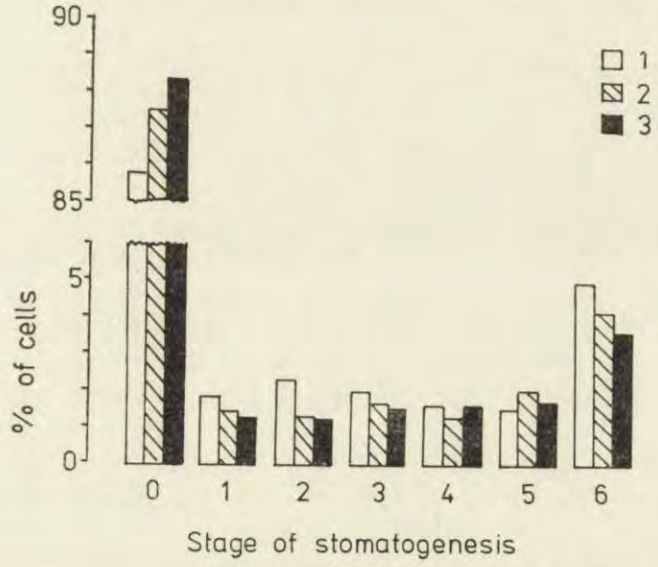

Fig. 3. The mean percentage of cells undergoing particular stages of stomatogenesis according to colistin concentration in the medium, *1* — control, *2* — colistin 0.05 mM/l, *3* — colistin 1 mM/l

Exposure to colistin at 1 mM/l induced resorption of the oral primordium in 4.5% of the ciliates after 45 min. After 2 h the primordium became resorbable in 6.5% of the organisms. Subsequently the number

of organisms revealing such a disorder in the process of stomatogenesis gradually decreased and no resorption was found after 12 h (Table 1, Fig. 4).

Since resorption of the oral primordium was not observed in normal exponentially growing *Tetrahymena*, the experiments might suggest that

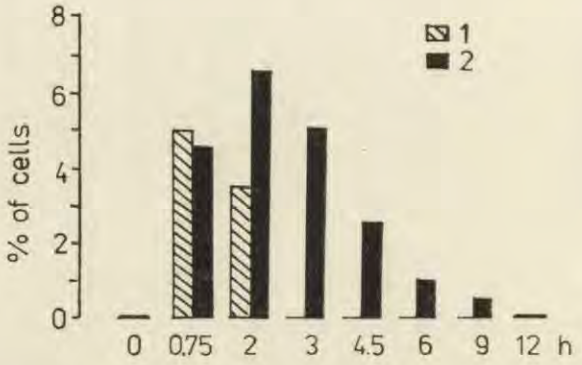

Fig. 4. The percentage of cells suffering resorption of the oral primordium according to colistin concentration in the medium and the duration of the experiment. No cells with resorption of the oral primordium were found in the control culture. N — 200 cells, *1* — colistin 0.05 mM/l, *2* — colistin 1 mM/l

the addition to the culture of colistin at given concentrations induces disorders in the process of stomatogenesis such as the resorption of the oral primordium developing in the opistore, as well as a temporary arrest of the new oral apparatus formation.

The origin of the cells which after a temporary arrest of stomatogenesis effected by the drug resume the process also seems a matter of importance. In the culture incubated with colistin at 0.05 mM/l the ciliates undergoing stomatogenesis (5.5%) appeared after 2 h, a corresponding count in the control sample being then 9%. The fraction of cells forming the oral primordia could have included organisms with the primordia undergoing resorption and as well as those with cells starting division morphogenesis. Since the increase in the number of ciliates undergoing particular phases of oral development between 45 min and 2 h was 5.5%, while the decrease in the number of organisms suffering resorption of the oral primordium was at that time 1.5%, the said fraction of *Tetrahymena* was likely to be derived mostly from the ciliates starting the process of cytokinesis. On the other hand, after 3 h the course of oral development in the culture taken from the investigated sample was the same as in the colistin-free culture. That was due to the fact that the cells entered the process of cytokinesis, while the organisms with the oral primordium undergoing resorption disappeared and thus

the number of cells undergoing various stages of stomatogenesis was the same in both the investigated sample and the control. To conclude, it may be said that in cultures treated with colistin at 0.05 mM/l the process of cytokinesis and the associated oral development occurred after 2 h, while the morphogenetic activation following resorption of the oral primordium appeared after 3 h of the ciliates exposure to polymyxin.

Addition of 1 mM/l colistin to the culture induced a blockage of oral development for 2 h when the ciliates were exposed to the drug. After 3 h there appeared a fraction of cells (1%) undergoing particular stages of stomatogenesis and it was impossible to establish the origin of these organisms. They could have been derived either from *Tetrahymena* beginning the process of oral development and therefore adapted to the said concentration of colistin, or from the ones suffering the resorption of the oral primordium. On the other hand, the emergence after 4.5 h of cells with the developing oral primordium making up 5.5% of the investigated population was due to the morphogenetic activation of those organisms which had resorbed the oral primordium and to the initiation of stomatogenesis and cytokinesis by some of the remaining ciliates.

Discussion

The effects of cell metabolism inhibitors upon the cell cycle have been the subject of numerous studies, giving rise to certain theories of its regulation (Mazia 1961, Zeuthen and Rasmussen 1971, Frankel and Williams 1973, Miyamato et al. 1973, Mitchison 1978).

(1) The cell cycle comprises a number of consecutive chemical syntheses. A blockage of one arrests the cycle until certain substrates reach the level required for the continuation of particular chemical processes.

(2) The cell cycle includes a few so-called critical moments. A blockage of one of the moments effects either a change in the course of the cycle or a reversal to some earlier stage, the latter phenomenon being known as a set-back.

The results obtained seem to suggest that in *Tetrahymena pyriformis* a continuous presence of colistin in the medium produces the cell cycle regulation of the second type, as demonstrated by resorption of the oral primordia in the ciliates cultured in the drug-containing medium. Studies on temperature-sensitive mutants of *Tetrahymena* have revealed that resorption of the developing oral structures is associated wtih a set-back rather than a mere "freezing" of the cycle at some stage (Frankel et al. 1980). The phenomenon of set-back also seems responsible

for the fact that at 1 mM/l colistin effects a smaller increase in the number of cells observed in a unit of time following the prolonged lag phase than is observed in the control sample. On the other hand, in the next stage of cultural growth, i.e., after 12 to 16 h, the two curves are virtually parallel. After the initial delay of divisions the percentage of cells undergoing various stages of oral development is significantly lower at the beginning of the process than the fraction of control cells completing their natural lag phase, which seems to be associated with the emergence of the phenomenon of resorption of the oral primordium. In this case colistin does not appear to be responsible for the "freezing" of cells at particular stages of their cell cycle. More likely, colistin effects resorption of the oral primordium in some cells and thus makes the fraction return to an earlier stage of the cell cycle with a subsequent increase in the fraction of morphostatic cells.

The phenomenon of oral primordium resorption has not been, so far explained. In their model of the formation of division protein Zeuthen and Williams (1969) have suggested that the emergence of a structure composed of a certain number of components is a prerequisite for cell division, two components at least being proteins. To produce the structure, one of the proteins must be continuously synthetized. The protein components, also called "division proteins", are linked in a linear manner into larger sub-units which are highly unstable intermediate structures. The components of such structure may be replaced, but if their synthesis is broken, the structure itself disintegrates. This very moment is particularly difficult to explain, and although the protein fraction has been isolated, which according to Watanabe and Ikeda (1965 a, b) meets the criteria for the division protein, the theory remains controversial.

The oral apparatus in *Tetrahymena* corresponds to the "division protein" as far as response to external agents is concerned (Mitchison 1978). The oral primordium differentiation may be reversed due to the arrest in protein synthesis, while the resorption of oral apparatus excludes any processes of cell division (Frankel 1964). The resorption of oral primordium in ciliates may follow two patterns.

(1) Resorption of the developing structures affects only a part of the oral apparatus, i.e., it is a "selective resorption of the "mounth-parts" during reorganization" (Tartar 1961).

(2) Resorption is an all-or-none process involving the entire oral primordium (Frankel 1967 b).

The obtained results seem to suggest that *Tetrahymena pyriformis* produces the resorption of oral apparatus of the second type.

The findings also demonstrate the stabilization point in the cell cycle of *Tetrahymena* with respect to colistin. Frankel (1967 c) has differentiated the following phases of oral development: 1 — 35 min, 2 — 8 min, 3 — 3 min, 4 — 20 min, 5 — 8 min and 6 — 15 min. Since after 45 min of exposure to antibiotic no dividing cells were observed, it means that those *Tetrahymena* which had already undergone the stabilization point in their cell cycle when colistin was added ended the processes of stomatogenesis and cytokinesis. Accordingly, the stabilization point must occur not later than 45 min prior to the completion of cell division, i.e., between stage 4 and stage 5 of oral development, including the phase of cytokinesis which lasts 18 min. Frankel (1962) has proved that temperature shock and *p*-fluorophenylalanine effect the stabilization point in the cell cycle of *Tetrahymena* early in stage 5 of oral development, the study being conducted on synchronized cells. Comparing his findings and the results of the present study, it may be concluded that the duration of particular stages of division morphogenesis is similar in both synchronized and asynchronized *Tetrahymena*. The shorter generation time in asynchronized ciliates (Mitchison 1978) seems to be due to the shorter period preceding the oral development (Buhse and Zeuthen 1974, Suhr-Jessen et al. 1977).

According to Frankel (1965), the resorption of oral primordium is brought about by a direct treatment of the cell with a protein or RNA synthesis inhibitor. The effect of antibiotic used in the present study has been thought to be limited to the cell membrane. However, the obtained results also suggest an indirect effect of the drug on the synthesis of the said compounds most probably, colistin does not directly block protein or RNA synthesis in *Tetrahymena*, but by increasing the cell membrane permeability causes a leakage from the cell of substrates necessary for the synthesis of these compounds. However, the drug does not reveal the effect typical of many detergents, since it does not produce ciliary reversal induced by potassium ions. This might be due to the lack of differences between the threshold KCl concentrations leading to a continuous ciliary reversal between cells from the control cultures and the ones containing colistin (Szablewski — in preparation).

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Receptor Level Study of Polypeptide Hormone (Insulin, TSH, FSH) Imprinting and Overlap in *Tetrahymena*

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Synopsis. Pretreatment of the *Tetrahymena* with polypeptide hormones (insulin, TSH, FSH) gave rise to positive imprinting for each adequate hormone, as judged from their enhanced binding on second exposure. Preexposure to insulin depressed the later binding of TSH and FSH, whereas preexposure to TSH or FSH had no influence on later insulin binding.

Like certain mammalian cells (Sandra et al. 1979), the unicellular *Tetrahymena* binds Concanavalin-A (Con-A), displays insulin-lectin overlap on its lectin binding sites (Csaba and Kovács 1982 a), and shows, on preexposure to insulin, an increased binding capacity for insulin and Con-A at reexposure (Kovács et al. 1984). Against this, preexposure to Con-A induces only a short-term imprinting for Con-A itself, and no imprinting whatever for insulin. Our pertinent studies on vertebrates and mammalian cells (Csaba 1981, Csaba et al. 1979, 1981, 1983 a,b, Csaba and Török 1983) have revealed a functional overlap of TSH and FSH on each other's binding sites, and have also shown that these related hormones (Pierce et al. 1976, Ward 1974) are able to induce binding sites for one another.

The *Tetrahymena* being a unicellular, it does not possess a hormonal system, although it does contain certain hormones which are characteristic of higher vertebrates (Le Roith et al. 1980). The membrane of the *Tetrahymena* may contain certain non-specific receptor-like structures which are capable of hormone binding (Csaba 1981), and the

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unicellular can therefore serve as a model organism in experimental studies on the formation of specific-appearing membrane receptors. With these facts in mind, we investigated the influence of pretreatment with Thyroid Stimulating Hormone (TSH), Follicle Stimulating Hormone (FSH) and insulin on the later binding of these hormones, to obtain information whether imprinting could occur in those receptor structures whose hormonal counterpart cannot, in principle, evoke a response in the unicellular for lack of target organs (cells), and to obtain evidence either for, or against, the existence in the *Tetrahymena* of a general peptide binding site, implied by earlier studies.

Materials and Methods

Tetrahymena pyriformis GL cells, maintained in 0.1% yeast extract containing 1% Bacto-tryptone medium (Difco, Michigan) at 28°C, were used in the logarithmic phase of growth. The cells were exposed to 10^{-6} M insulin (Insulin Semilente MC, Novo, Denmark), 1.2 IU/ml TSH (Ambinon, Organon Oss) or 0.6 IU/ml FSH–LH; Pergonal, HUMAN, Budapest — Serono, Rome) for 1 h.

After treatment, the cells were washed in culture medium, returned to plain medium for another day, fixed in 4% neutral formaline (in pH 7.2 PBS) for 5 min at room temperature, washed in three changes of PBS, and incubated in presence of FITC-labeled hormone.

The FITC : protein ratio and protein content of the labeled hormones were the following: FITC-insulin 0.24, 0.4 mg/ml; FITC–TSH: 1.39, 0.4 mg/ml; FITC–FSH: 1.88, 0.4 mg/ml.

After incubation with FITC-labeled hormone, the cells were washed in three changes of PBS, spread on slides, dried, and examined for intensity of fluorescence with a Zeiss Fluoval cytofluorimeter, connected with a Zeiss MFV 4001 amplifier for amplification of the signals, a digital processor for signal transformation, and a Hewlett Packard 41 C calculator for assessment of mean values and standard deviation, and evaluation of the results by analysis of variance and Student's

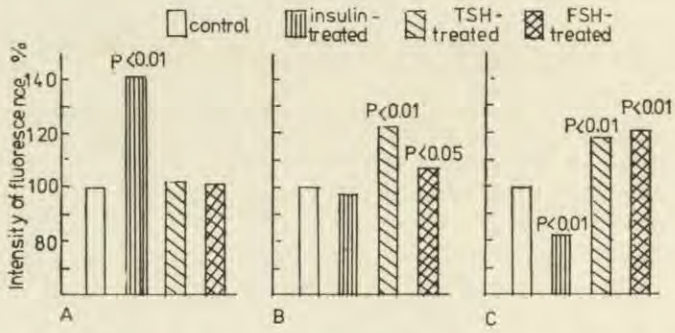

Fig. 1. Binding of FITC labeled insulin (*A*), TSH (*B*) and FSH (*C*) to *Tetrahymena*

t-test. Twenty cells were assayed in each group, and each assay was performed in four replicates, thus the mean values found pertain to 80 cells in each group. The results (of similar tendency) are shown in Fig. 1.

Results and Discussion

In previous studies we were able to induce overlapping binding sites in the *Tetrahymena* with several biologically active materials. Antibodies to rat hepatocellular receptor and insulin provoked the formation of binding sites, which were capable of intensive interaction with both materials (Csaba et al. 1983 a). In the case of insulin and Con-A, only the former was capable of forming binding sites for Con-A, whereas the latter formed binding sites only for itself, and for a short term. A similar tendency to overlap was demonstrated with histamine and Con-A (Csaba and Kovacs 1982 b).

The present experimental observations have shown that pretreatment with insulin gave rise to a considerable increase (42% over the control) in the FITC-insulin binding capacity of the *Tetrahymena* (Fig. 1), whereas it did not appreciably alter the binding relations of TSH and FSH.

Pretreatment with TSH accounted for a significant increase in both TSH and FSH binding capacity over the control. Pretreatment with FSH caused a highly significant ($P < 0.01$) increase in FSH-binding, and a less significant ($P < 0.05$) increase in TSH-binding.

The FITC-insulin binding capacity of the cells pretreated with TSH or FSH did not significantly differ from the control.

It appears that the above pretreatments either induced the formation of specific receptors or, supposing that non-specific receptor structures were integral parts of the membrane, caused the amplification of the latter for the adequate hormone.

Functional overlaps of TSH and FSH were also demonstrated in mammalian cell cultures.

The effect of preexposure to TSH and FSH differed nevertheless to a certain degree (to the advantage of TSH), indicating that, like mammalian cells, the unicellular *Tetrahymena* is capable of structural differentiation between TSH and FSH.

The question may be posed whether in this light the earlier hypothesis (Csaba and Kovács 1982 a, Lenhoff 1974) that lower organisms including the *Tetrahymena* possess a general — primitive — peptide receptor, still holds, since if such a receptor existed, preexsposure to insulin would have amplified the receptors for TSH and FSH, and vice versa. However, the problem is presumably not as simple as that.

Probably the general peptide binding ability of the membrane-associated polypeptide-sugar (receptor) structures becomes abolished by the first interaction with the adequate (specific) hormone, which renders the interacting membrane structure specific by amplifying it for itself and only for itself. This hypothetical implication accords well with the experimental fact (Kovacs et al. 1984) that while insulin (as a fully specific hormone) amplified the receptor for itself and Con-A, the latter, being a sugar-specific, but not receptor-specific lectin, failed to amplify the receptor for insulin.

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Haemoproteus rupicola sp. n. from the Hill-stream Fish *Noemacheilus rupicola rupicola* (McClelland) of Darjeeling Area

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Synopsis. The communication deals with the morphology and taxonomy of *Haemoproteus rupicola* sp. n. from a hill-stream fish *Noemacheilus rupicola rupicola* (McClelland). Only the gametocytes have been found in the red cells and rarely in leucocytes. The macrogametocytes measure 3.9 μm by 1.95 μm on the average. The microgametocytes measure 4.3 μm by 2.4 μm. This is the first report of a *Haemoproteus* from a piscine host.

In a place like Darjeeling, on a high altitude, there are innumerable Himalayan streams with chains of fresh water puddles. Different types of fishes are found in these waters. In the course of investigation on protozoan parasites of hill-stream fishes in and around Darjeeling the authors encountered a pigmented haemoprotozoan which has been identified as *Haemoproteus rupicola* sp. n. in *Noemacheilus rupicola rupicola* (McClelland). The host fish is locally known as "Garella".

Materials and Methods

The fishes were collected from the streams (Jhora) of the area Chowkiderdara of Pandam Tea Estate, Darjeeling (altitude 1600 m MSL). The blood smears were prepared from the blood obtained by cutting the tail end of the fishes. The smears were air-dried, fixed in methanol, stained with Romanowsky stain (Giemsa and Leishman) and differentiated with neutral distilled water and were examined under oil-immersion lens. Measurements were obtained from the camera lucida drawings drawn on a graph paper (mm division), as it ensured the area measurement by counting the squares covered. To obtain morphometric parameters Ben-

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nett and Campbell (1972) was followed. Photomicrographs and line drawings were done with a Leica M4-2 camera with photomicrographic attachment and Leitz camera lucida respectively.

Results

Only the gametocytes of *H. rupicola* sp. n. have been encountered in the peripheral blood.

Description:

Immature gametocyte: (Fig. 1 *A*) N = 10. These are intracorpuscular, slightly elongated parasites measuring 3.0 to 4.5 μm (average 3.9 μm) in length and 1.5 to 2.5 μm in width (average 1.95 μm). The body area measures 6.2 μm^2 on the average with a range of 2.75 to 7.75 μm^2. The cytoplasm is blue. Pigments are finely granular and black in colour. Nucleus is deeply stained, situated centrally, measuring 0.5 to 1.5 μm in diameter with a mean area of 1.04 μm^2.

Microgametocyte: (Fig. 1 *B* and Pl. I A) N = 25. These are oval shaped parasites measuring 3.5 to 6 μm in length (average 4.3 μm) and the width varies from 2 to 3.5 μm with an average of 2.4 μm. The body area ranges from 6 to 12.25 μm^2 (average 8.47 μm^2). The parasites are situated at one pole of the host cell. The cytoplasm stains lighter in its central area than the peripheral part. Pigments in the form of small dots, black coloured and distributed at the periphery of the parasite

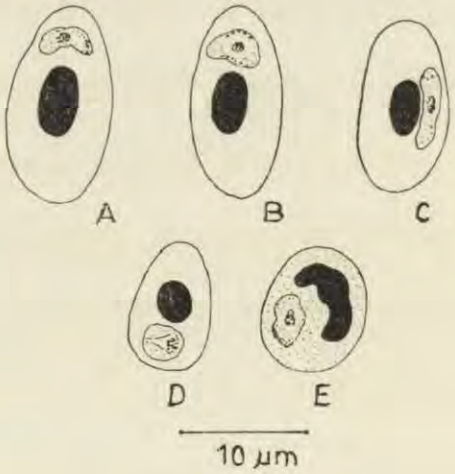

Fig. 1. (*A–E*) Camera lucida drawings of *Haemoproteus rupicola* sp. n.; *A* — Immature gametocyte, *B* — Microgametocyte, *C* — Macrogametocyte, *D* — A gametocyte in an early erythrocyte, *E* — A gametocyte in a leucocyte

body. Nucleus is fragmented and less compact, measuring 0.5 to 1.5 μm in diameter (average 1.06 μm). It stains faint purple with Leishman stain.

Macrogametocyte: (Fig. 1 *C* and Pl. I B) N = 30. These are slightly curved, situated at one side of the red cell facing its concave side towards the convex margin of the host cell nucleus. The cytoplasm is homogenous and stains deep blue. Pigment granules are oriented in the same manner as in male gametocytes. The parasite measures 3.5 to 6.5 μm (average 4.9 μm) by 1.5 to 3 μm (average 1.81 μm). The body area measures 3.5 to 10.5 μm^2 with an average of 6.68 μm^2. The diameter of the nucleus measures 1.34 μm on the average.

The parasites usually infect the matured red cells, but often they are found to infect the early erythrocytes (Fig. 1 *D*) and rarely leucocytes (Fig. 1 *E* and Pl. I C). A comparative assessment of length, width, area and NDR (Nucleus displacement ratio) of the uninfected red cells reveals no significant difference. However, in some cases the nucleus of the infected host cell is slightly displaced in presence of the parasite.

Vector and tissue stages are under investigation and will be communicated elsewhere in due time.

Type host: *Noemacheilus rupicola rupicola* (McClelland)
Type locality: Pandam Tea Estate, Darjeeling
Localization: Blood cells.

Diagnosis of *Haemoproteus rupicola* sp. n.: Gametocytes mostly in the red cells. Macrogametocytes measure 3 to 4.5 μm (average 3.9 μm) by 1.5 to 2.5 μm (average 1.95 μm). Microgametocytes measure 3.5 to 6 μm (average 4.3 μm) by 2 to 3.5 μm (average 2.4 μm). Pigments distributed in the peripheral area. Gametocytes may not always keep the host cell nucleus in their concavity.

Discussion

The occurrence of only gametocytes and the absence of trophozoites or schizont in the blood film, the presence of typical pigments, the shape and nuclear position of the haemoprotozoan under report justify its inclusion under the genus *Haemoproteus*. The parasite under discussion does not always follow to keep the host cell nucleus in its concavity. In this point a slight deviation from the common generic characters of *Haemoproteus* is noted. At the same time it appears from the available literature (Ray and Choudhury 1981) that this particular character is not maintained universally by *Haemoproteus* except the avian forms. Identification of micro- and macrogametocytes has been done

following Ray and Choudhury (1981). A perusal of literatures reveals that there are no reports of *Haemoproteus* from the cold blooded animals except those by Fantham et al. (1942) and Ray and Choudhury (1981), who reported this genus from *Bufo americanus* and *Rana limnocharis* respectively. Neither Levine and Campbell (1971) in their "Check-list of the species of the genus *Haemoproteus* (*Apicomplexa, Plasmodiidae*)" nor Nandi et al. (1983) in the "Index catalogue and bibliography of protozoan parasites from Indian fishes" have mentioned any occurrence of the genus *Haemoproteus* from piscine host. It appears to be the first report of a *Haemoproteus* from the fish host. On the other hand a report of any haemoprotozoa from *N. rupicola rupicola* is also being done for the first time from this country. The authors are aware of the reluctancy of some of the authorities to describe a new species of parasite without studying its full life cycle and cytophysiological characteristics. At the same time they are inclined to think (Bennett and Campbell 1972) that a mere reference to a morphologically definable taxon by generic designation only would ignore other important attributes for consideration such as distribution, host specificity etc. Moreover, the parasite under report does not fit any other known species of the genus and the authors propose a new species designation as *Haemoproteus rupicola* sp. n. The species name is given after the specific name of the host fish. The type specimens will be deposited at the National Collection of Zoological Survey of India, Calcutta.

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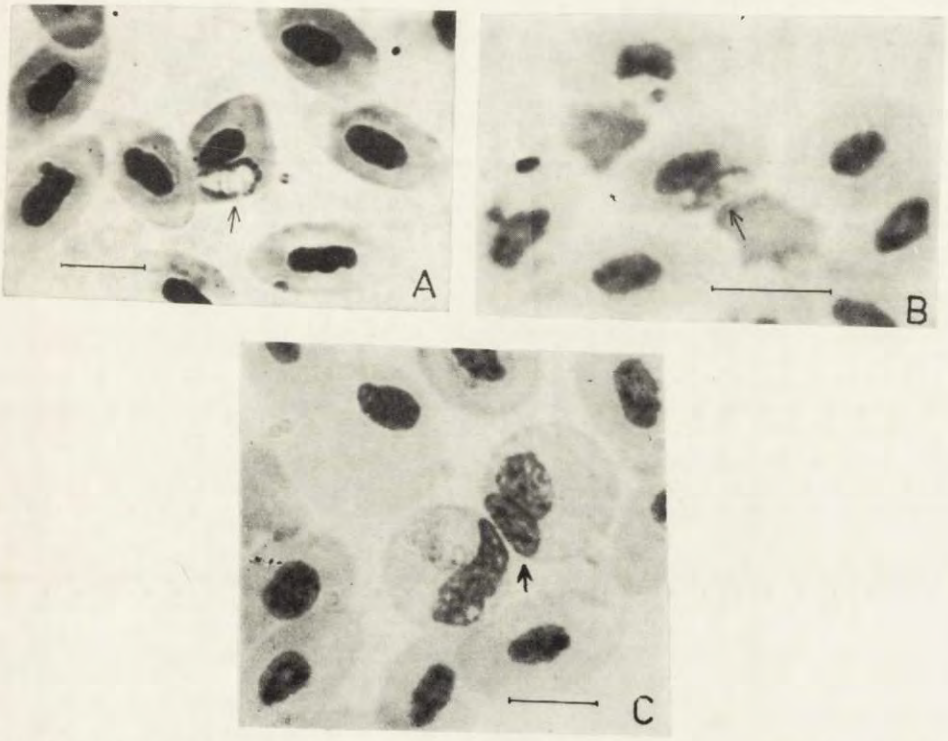
EXPLANATION OF PLATE I

Photomicrographs of *H. rupicola* sp. n.

A: Microgametocyte
B: Macrogametocyte
C: A gametocyte in a leucocyte
(Scale: 10 μm)



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Some Coelozoic Myxosporidans (*Myxozoa* : *Myxosporea*) from a Fresh Water Teleost Fish of River Padma

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Synopsis. Three coelozoic myxosporidans (*Myxozoa* : *Myxosporea*) viz.; *Thelohanellus wallagoi* sp. n. and *Myxosoma bhaduria* sp. n. from the gall bladder and *Myxobolus attui* sp. n. from the gut of a fresh water fish *Wallago attu* Bleeker of the river Padma now in Bangladesh, have been described. The three species have also been compared with their closely related, previously described species in order to establish their distinctiveness.

The present paper describes three coelozoic myxosporidans from a fresh water teleost fish, while the investigation was carried out to study the parasitic protozoa of the fishes of the river Padma now in Bangladesh, during the period of 1981–1983.

Material and Methods

The fishes were collected in fresh condition from the fishermen of the east bank of the river Padma, kept in ice bucket and brought back to the Islampur camp where they were thoroughly examined under a microscope for myxosporidan parasites. The parasites were studied in the college laboratory at Naihati from wet smears treated with Lugol's iodine solution and also from dry smears stained with Giemsa after fixation in absolute methanol (the slides were prepared at the Islampur camp) under the oil immersion lens of an Olympus research microscope. The extrusion of polar filament was achieved with 2.5% KOH solution. The line drawings were made with the help of a camera lucida. The measurements are given in micrometer (μm).

Observations

Thelohanellus wallagoi sp. n.

Cyst and other vegetative forms: Not found.

Spore: The spores were pyriform in valvular view with anterior pointed and posterior rounded ends. The anterior extremity was truncate (Fig. 1 *B,C,D*). In sutural view it was lenticular with slightly curved suture, not ridged (Fig. 1 *A*). The shell valve was thick-walled and smooth. The polar capsule one, was slightly excentric, anteriorly placed, elongately pyriform with 4–5 coils of polar filament (Fig. 1 *B*). The extruded polar filament was 20 μm long (Fig. 1 *D*). The extracapsular space of the spore was filled with finely granular uninucleate sporoplasm. The iodinophilous vacuole was absent.

Dimensions (mensural range of 38 fresh spores with mean within parenthesis is given below):

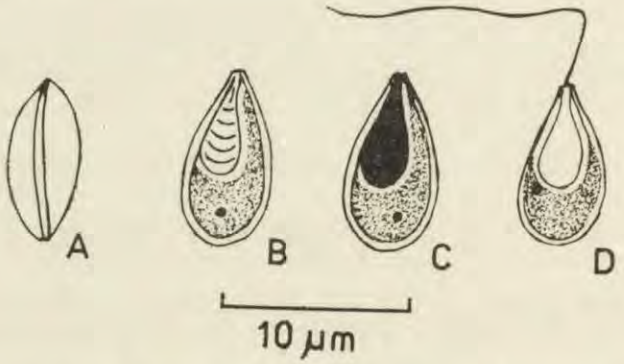

Fig. 1. Spores of *Thelohanellus wallagoi* sp. n., *A* — A fresh spore in sutural view, *B* — A spore in valvular view — treated with Lugol's iodine solution, *C* — A spore stained with Giemsa, *D* — A spore showing extruded polar filament

Spore length — 8.0–11.2 (9.25)
Spore width — 4.0–6.4 (4.85)
Polar capsule length — 4.5–6.4 (5.47)
Polar capsule width — 2.5–3.0 (2.71)
Infection locus: Gallbladder
Incidence: two positive out of 16 examined
Host: *Wallago attu* Bleeker
Locality: East bank of Padma river, West Bengal, India

Myxobolus attui sp. n.

Cyst and other vegetative forms: Not seen.

Spore: The spores were coelozoic, oval to ellipsoidal with slightly compressed anterior end in valvular view (Fig. 2 *B*) and almost lenticular

to fusiform in sutural view (Fig. 2 *A*). The shell valves were thick-walled and smooth with thick, slightly curved suture. The two polar capsules were equal, pyriform with moderately long tubular neck opening anteriorly, almost parallel or slightly convergent (Fig. 2 *B*, *C*). Each capsule was housed in a pericapsular space with 6–7 coils of polar filament. The polar filament when extruded was 33.6 μm long. The large extracapsular space was filled with finely granular binucleate sporoplasm. The sporoplasm had a large, spherical iodinophilous vacuole in it (Fig. 2 *B*).

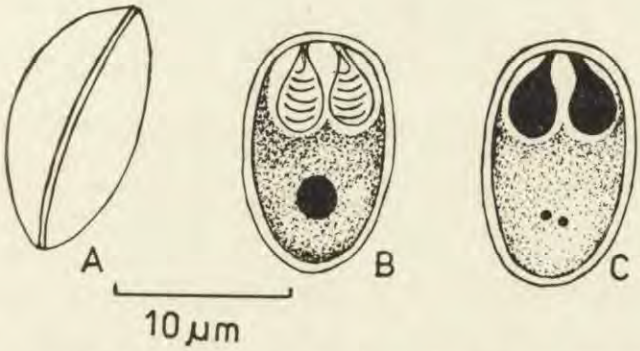

Fig. 2. Spores of *Myxobulus attui* sp. n., *A* — A spore in sutural view, *B* — A spore in valvular view — treated with Lugol's iodine solution, *C* — A spore stained with Giemsa

Dimensions (mensural range of 18 fresh spores with mean within parenthesis is given below):

Spore length — 12.8–15.2 (13.89)
Spore width — 7.5–9.6 (8.53)
Polar capsule length — 4.8–7.2 (5.92)
Polar capsule width — 2.4–4.0 (3.00)
Infection locus: Gut
Incidence: two infected out of 16 examined
Host: *Wallago attu* Bleeker
Locality: East bank of Padma river, West Bengal, India

Myxosoma bhaduria sp. n.

Vegetative forms: Not seen.

Spore: The spores were coelozoic, lenticular in sutural view (Fig. 3 *A*), pyriform in valvular view (Fig. 3 *B* and *C*). The anterior end was broadly pointed and the posterior end was round. The shell valves were thin-walled and smooth with thin, non-ridged suture. The two polar capsules were elongately pyriform, equal and convergent anteriorly; they opened side by side but never crossed. Each polar capsule

contained 5–6 coils of polar filament (Fig. 3 *B*). The extracapsular cavity was filled with a semicircular mass of coarse, binucleate sporoplasm. The capsulogenous and the valvugenous nuclei were not distinct. The iodinophilous vacuole in the sporoplasm was absent.

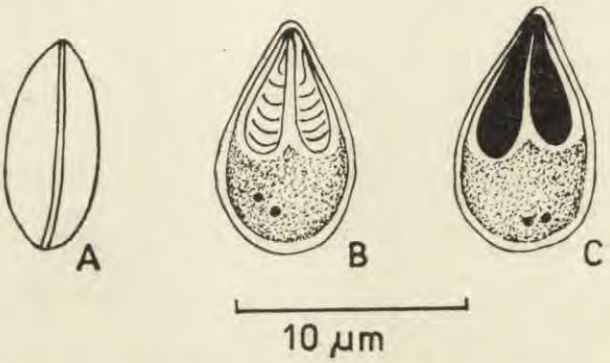

Fig. 3. Spores of *Myxosoma bhaduria* sp. n., *A* — A fresh spore in sutural view, *B* — A spore in valvular view — treated with Lugol's iodine solution, *C* — A spore stained with Giemsa

Dimensions (mensural range of 22 fresh spores with mean within parenthesis is given below):

Spore length — 8.8–11.2 (10.59)
Spore width — 4.8–6.72 (6.28)
Polar capsule length — 4.0–6.4 (5.31)
Polar capsule width — 2.4–3.2 (2.78)
Infection locus: Gallbladder
Incidence: four infected out of 16 examined
Host: *Wallago attu* Bleeker
Locality: East bank of river Padma, West Bengal, India.

Discussion

So far, 12 species of *Thelohanellus* Kudo, 1933 (Kalavati et al. 1981), 18 species of *Myxobolus* Butschlii, 1882 (Kalavati et al. 1981) and 10 species of *Myxosoma* Thelohan, 1892 (Haldar et al. 1981, Kalavati et al. 1981 and Richard et al. 1981) have been reported from Indian fresh water fishes. The present species of *Thelohanellus* Kudo closely resembles *T. calbasui* Tripathi, 1951 reported from the scales of *Labeo calbusu* Ham. in the length and shape of its spore, *T. boggoti* Qadri, 1962 reported from the gills of *Labeo boggot* Ham. in the range of its spore length, and *T. chelae* Lalitakumari, 1969 reported from the bile of *Chela bacaila* Ham. in the range of its spore length and

in the same infection locus. However, the present myxosporidan differs from *T. calbasui* by narrower width of its spore and polar capsule, different infection locus and widely different host fish. It differs from *T. boggoti* by shorter length and narrower width of its spore besides its different infection locus and host fish. It is also distinct from *T. chelae* by the longer dimension of its spore and its widely different host fish (Table 1). The myxosporidan is, therefore, considered as a new species, to which the name *Thelohanellus wallagoi* sp. n. is given after the name of its host.

Table 1

Comparative study of *Thelohanellus wallagoi* sp. n. with its related *Thelohanellus* spp.

Parasite (Host)	Infection locus	Spore		Polar capsule	
		Range	Mean	Range	Mean
T. wallagoi sp. n.	Gallbladder	8.0–11.2	9.25[a]	4.5–6.4	5.47
(*Wallago attu* Bleeker)	(bile)	4.0–6.4	4.85[b]	2.5–3.0	2.71
T. calbasui Tripathi, 1951	Scales	9.0–10.8	—	—	5.4
(*Labeo calbasu* Ham.)		—	7.2	—	3.4
T. boggoti Qadri, 1962	Gills	11.0–12.0	—	—	—
(*Labeo boggot*)		6.0–7.5	—	—	—
T. chelae Lalitakumari, 1969	Bile	9.4–10.3	—	—	—
(*Chela bacaila* Ham.)		4.6–6.0	—	—	—

[a] length, [b] width.

The myxosporidan of *Myxobolus* Butschlii closely resembles *M. calbasui* Chakravarty, 1939 reported from the gallbladders of *Labeo rohita* Ham., *L. calbasu* Ham. and *Cirrhina mrigala* Ham., *M. osmaniae* Lalitakumari, 1969 reported from the liver and intestine of *Barbus punaubensis* and *M. punctatus* Ray-Choudhury and Chakravarty, 1970 reported from the spleen and pharyngeal epithelium of *Channa punctatus* Bl. in the dimension of its spore length. However, the present myxosporidan differs from *M. calbasui* in having anteriorly compressed ellipsoidal spore and equal polar capsules (roughly oval spore with sharply pointed anterior end and unequal polar capsules in *M. calbasui*). It separates itself from *M. osmaniae* in having narrower width of its spore and different host. The parasite also differs from *M. punctatus* by its wider spore, distinct infection locus and different host (Table 2). The myxosporidan has been, therefore, regarded as a new species and the name *Myxobolus attui* sp. n. is proposed after the specific name of its host.

The present species of *Myxosoma* Thelohan shows similarity with *M. hyderabadense* Lalitakumari, 1969 reported from the gills of *Barbus*

Table 2

Comparative study of *Myxobolus attui* sp. n. with its related *Myxobolus* spp.

Parasite (Host)	Infection locus	Spore		Polar capsule	
		Range	Mean	Range	Mean
M. attui sp. n.	Gallbladder	12.8–15.2	13.89[a]	4.8–7.2	5.92
(*Wallago attu* Bleeker)	(bile)	7.5–9.6	8.53[b]	2.4–4.0	3.00
M. calbasui Chakravarty, 1943	Same	12.4–15.0	—	—	6.18
(*Labeo calbasu* Ham. *L. rohita*		8.2–10.0	—	—	4.12
				and	
Ham. *Cirrhina mrigala* Ham.)				—	4.12
				—	3.09
M. osmaniae Lalitakumari 1969	Liver and	12.0–15.0	—	—	—
(*Barbus punaubensis*)	intestine	7.1–10.0	—	—	—
M. punctatus Ray-Choudhury and Chakravarty, 1970	Spleen and pharyngeal	12.3–15.0	14.45	8.6–10.0	9.29
(*Channa punctatus* Bl.)	epithelium	5.7–7.86	6.67	2.1–2.86	2.62

[a] length, [b] width.

pinnarautus, *M. dermitis* Haldar et al. (1981) reported from the scales of *Labeo rohita* Ham. and *M. magauddi* Richard et al. (1981) reported from the gill filaments of *Trichogaster fasciatus* Bl. Schn. in having closely related length-range of the spore. However, the spore of the present species is narrower in width-range than the former three myxosporidan spores. Moreover, the polar capsule of the present form is equal (unequal in *M. magauddi*), larger than *M. dermitis* and smaller than *M. hyderabadense* (Table 3). The present parasite is, therefore, de-

Table 3

Comparative study of *Myxosoma bhaduria* sp. n. with its related *Myxosoma* spp.

Parasite (Host)	Infection locus	Spore		Polar capsule	
		Range	Mean	Range	Mean
M. bhaduria sp. n.	Gallbladder	8.8–11.2	10.59	4.0–6.4	5.31
(*Wallago attu* Bleeker)	(bile)	4.8–6.7	6.28	2.4–3.2	2.78
M. hyderabadense Lalitakumari, 1969	Gills	9.3–11.5	10.1	5.0–7.3	5.8
(*Barbus pinnarautus*)		5.0–8.0	5.9	1.4–3.0	2.2
M. dermitis Haldar et al. 1981	Scales	9.0–11.0	10.3	4.0–5.0	4.4
(*Labeo rohita* Ham.)		8.0–10.0	9.4	2.0–3.0	2.2
M. mugauddi Richard et al. 1981	Gill filaments	10.8–11.7	11.2	3.3–5.0	4.0
(*Trichogaster fasciatus* Bl. Schn.)		8.3–10.0	9.2	2.5–3.3	3.0
				and	
				2.5–3.3	2.6
				1.7–2.5	1.8

scribed as a new species and the name *Myxosoma bhaduria* sp. n. is proposed after late Prof. Dr. J. L. Bhaduri of the University of Calcutta. cutta.

Material: Syntype specimens of *Thelohanellus wallagoi* sp. n. and *Myxosoma bhaduria* sp. n. prepared from the gall bladder and *Myxobolus attui* sp. n. prepared from the gut of *Wallago attu* Bleeker of the river Padma, on slides No., MXT-8 MXMs-5 and MXMb-12 respectively, are kept in the Department of Zoology, R. B. C. College, Naihati and soon will be deposited at the National Collection of the Zoological Survey of India, Calcutta.

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Myxosporidan *Henneguya mystusia* sp. n. (*Myxozoa* : *Myxosporea*) from the Gill of a Fresh Water Teleost Fish *Mystus* sp.

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Received on 18 July 1984

Synopsis. A new myxosporidan *Henneguya mystusia* sp. n. (*Myxozoa* : *Myxosporea*) is described from the gill filaments of a fresh water teleost *Mystus* sp. of West Bengal, India. Its morphometric data (mean) are: total length — 32.3, spore — 13 × 3.75 × 2.87, polar capsule — 5.04 × 1.2, caudal appendage — 19.33 and iodinophilous vacuole — 1.6.

During an investigation on parasitic protozoa of fresh water fishes in West Bengal, India, a myxosporidan parasite has been found to infect the gill filaments of *Mystus* sp. A description of the parasite under the genus *Henneguya* Thelohan, 1892 has been incorporated here.

Material and Methods

All autopsies were performed from live fishes collected from fishermen of Chinsurah, West Bengal, India. Fresh wet smears of the myxosporidan spores treated with Lugol's iodine solution and dry smears stained with Giemsa after fixation in absolute methanol were examined under 100 × 15 magnification of the Olympus research microscope. The figures have been drawn with the aid of a camera lucida. All the measurements are given in micrometer (μm).

Observations

Henneguya mystusia sp. n.

Description:

Cyst: A few small, ovoidal, opaque-white cysts were found on the gill filaments; the dimension of the largest cyst was 0.2 × 0.1 mm. The cysts contained mature spores only, polysporous.

Spore: The mature spores were fusiform in valvular view, with widest region just posterior to the polar capsule (Fig. 1 *B*, *C*); obovate in sutural view (Fig. 1 *A*) anterior end was very slightly curved and blunt; suture was thin, straight to very slightly curved, dividing the spore body into two almost equal parts. The spore wall was smooth and thin, composed of two smooth, symmetrical shell valves, each tapering posteriorly into a very long and fine caudal prolongation. The two polar capsules were tubular and almost equal, slightly converged anteriorly; the polar filament was not very distinct. The extra-capsular spore cavity was filled with finely granular sporoplasm containing a small spherical, orange colour iodinophilous vacuole (Fig. 1 *B*) and two sporoplasm nuclei (Fig. 1 *C*).

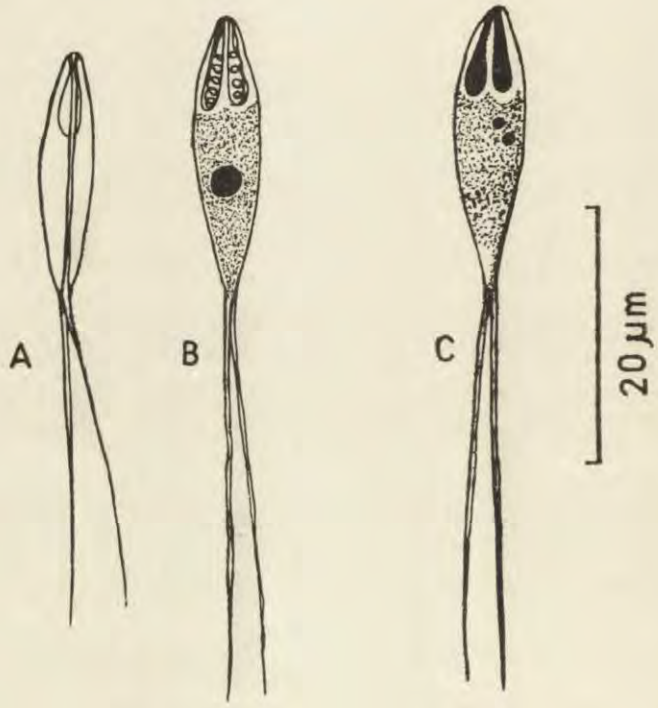

Fig. 1 *A–C*. Spores of *Henneguya mystusia* sp. n. *A* — A fresh spore in sutural view, *B* — A fresh spore in valvular view treated with Lugol's iodine, *C* — A spore in valvular view — stained with Giemsa

Dimensions: (Based on 36 fresh spores; mean value is given with range within the parenthesis):

Total length — 32.33 (27.0–40.0)
Length of spore — 13.0 (12.0–15.0)
Width of spore — 3.75 (3.0–4.0)

Thickness of spore — 2.87 (2.5–3.0)
Length of polar capsule — 5.04 (5.0–6.0)
Width of polar capsule — 1.2 (1.0–1.3)
Length of caudal prolongation — 19.33 (17.0–25.0)
Diameter of iodinophilous vacuole — 1.6 (1.5–1.8)
Infection locus: Gill filament
Incidence: one infected out of 15 examined
Pathogenicity: Not apparent
Host: *Mystus* sp.
Locality: Chinsurah, West Bengal, India
Time: 3rd week of June, 1984

Remark: Altogether 9 *Henneguya* spp. have been recorded from Indian fresh water fishes (Kalavati et al. 1981). Among them, *H. latesi* Tripathi, 1951 reported from the gills of *Lates calcarifer* resembles the present species by the total length-range of its spore (26.2–36.2 total length-range in *H. latesi*). The reported species also resembles *H. singhi* Lalitakumari, 1969 reported from the gill content of *Notopterus osmanae* in having closely related length-range of its spore (11.1–13.6 length-range in *H. singhi*). But the latter species is wider than the present form (3.9–5.7 spore-width in *H. singhi*). Moreover, the reported myxosporidan shows similarity with *H. waltairensis* Narasimhamurti and Kalavati, 1975 reported from the gills of *Ophicephalus punctatus* Bl. in having closely related morphometry of the spores (14.6–16.5 × 3.2–4.0 in *H. waltairensis*). However, the former myxosporidan is very distinct from the latter species by its fusiform spore (oval spore in *H. waltairensis*) and almost straight and shorter caudal prolongation (U-shaped and 40.0–50.0 μm long caudal prolongation in *H. waltairensis*). Besides, its fusiform spore is also comparable with *H. exilis* Kudo, 1929 and *H. longicaudata* Minchew, 1977 reported from the gills of *Ictalurus punctatus* (Rafinesque). However, the myxosporidan in the present study differs from the latter two species by the morphometric variations of spore (spore length and total length of *H. exilis* are 18.0–20.0 and 60.0–70.0 and those of *H. longicaudata* are 14.0–17.5 and 70.0–110.0). The myxosporidan under consideration is, therefore, believed to be a new species for which the name *Henneguya mystusia* sp. n. is proposed after the name of the host.

Material: Syntypes specimens on slide No. MHX-9, prepared from the gills of *Mystus* sp. on 16 June, 1984 have been deposited in the Department of Zoology, R. B. C. College, Naihati and soon will be sent to the National Collection of the Zoological Survey of India, Calcutta.

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Observations on the Infectivity of *Trypanosoma brucei* to Domestic Ducks

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Synopsis. In vitro sensitivity testing showed that normal duck serum is not trypanocidal to four variable antigen types of *Trypanosoma brucei*. Intravenous inoculation of $10^{7.8}$ mouse infective organisms (ID_{63}) of one variable antigen type failed to infect domestic ducks. Inoculation of a cocktail of three variable antigen types containing $3 \times 10^{7.8}$ organisms produced a non-persistent infection. Sera from trypanosome infected ducks agglutinated erythrocytes from rabbits, guinea pigs and rats. These observations suggest that the immune response is intimately involved in the susceptibility of domestic ducks to infections by *Trypanosoma brucei* and that the test of heterophile antibody might be used in detecting trypanosome-incontact ducks.

Trypanosoma brucei has the broadest potential host range and has been able to establish itself in representatives of apparently all orders of mammals (Hoare 1972), domestic chickens (Corson 1931, Zillmann and Mehlitz 1979) and even reptiles (Woo and Soltys 1969). There are, however, conflicting reports about the susceptiblity of domestic ducks to salivarian trypanosome. Seager (1944) reported a fatal experimental infection by *Trypanosoma equiperdum* in ducks following syringe inoculation of rat infected blood. On the other hand Duke (1912) reported that domestic ducks are insusceptible to *Trypanosoma brucei* infection. In a test on the infectivity of three stacks of *Trypanosoma brucei* to domestic chickens Joshua et al. (1982) found that one stock (Lugala/55/EATRO/459) regularly produced chronic infections in domestic chickens while the other two stocks failed to produce a persistent infection. These observations suggest that the infec-

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tivity of *Trypanosoma* (*Trypanozoon*) *brucei* to domestic chickens in general and to domestic ducks in particular is a liabile character.

All available citations of salivarian trypanosome infections in domestic ducks were carried out with uncloned populations. It is therefore necessary to reevaluate the infectivity of *Trypanosoma brucei* to ducks by using defined populations which are derived from clones of known viability. Laboratory observations on attempts to induce *T. brucei* infections in domestic ducks are reported in this paper.

Materials and Methods

Trypanosoma brucei (Primary isolation number Lugala/55/EATRO/459). This stock was first isolated from wild-tsetse flies at Uganda in 1955. Full pedigree of this stock has been published by Herbert and Parratt (1979).

The trypanosomes were kept cryopreserved as stabilates, identified by the prefix WIG (Western Infirmary Glasgow). Cloned populations of differing variable antigen types (VATs) isolated from this stock were denoted by the prefix WITat (Western Infirmary *Trypanozoon* antigen type). These clones produce persistent infections lasting over one year in domestic chickens (Joshua et al. 1978, 1982).

Variable antigen types WITat 1, WITat 2, WITat 3 and WITat 4, all from the same serodeme, were used in this investigation. Ducks — Adult male Aylesbury cross were used. The ducks were one and a half years old by the time they were inoculated with trypanosomes. These birds were kept in a deep litter system and were fed on duck pellets. No vaccinations against any infectious diseases were given to these birds. Mice — Adult (24+ weeks) Balb/C mice were used for animal inoculation experiment. Rat erythrocytes for heterophile antibody tests were obtained from male adult Charles river rats.

Rabbit erythrocytes were obtained from male, five-months-old New Zealand white.

Guinea pig erythrocytes were obtained from male six-month-old Dunkin Hartley breed. Trypanosome inoculation into and blood collection from the ducks were made in the brachial vein.

In vitro test

A preliminary experiment was carried out to test *in vitro* trypanocidal action of duck serum on *Trypanosoma brucei brucei*. *Trypanosoma brucei*, WITat 1 (Stabilate WIG 76) was retrieved from the cryobank and rapidly thawed. The contents of the capillary tube were diluted in 0.6 ml of normal saline and 0.3 ml of the suspension was incubated with 2 ml of duck serum at 37°C for 2 h. A duplicate experiment was carried out with normal rat serum, seeded with 0.3 ml of the diluted stabilate, similar tests were repeated using WITat 2, WITat 3 and WITat 4 (Table 1).

Mice in groups of five were each inoculated intraperitoneally with 0.1 ml of one suspension. The blood of the inoculated mice was monitored for parasitaemia for twenty days by examination of tail blood under the ordinary light microscope.

Table 1

Results of *in vitro* trypanocidal effect of duck serum on four variable antigen types of *T. brucei*

Trypanosomes variable antigen types	Number of organisms incubated per ml of duck serum	Estimated[1] organisms inoculated per animal	Number infected/Number inoculated
WITat 1	$10^{5.1}$	$10^{4.1}$	5/5
	$10^{4.1}$	$10^{3.1}$	5/5
	$10^{3.1}$	$10^{2.1}$	4/5
	$10^{5.8}$	$10^{4.8}$	5/5
WITat 2	$10^{4.8}$	$10^{3.8}$	5/5
	$10^{3.8}$	$10^{2.8}$	5/5
WITat 3	$10^{5.6}$	$10^{4.6}$	5/5
	$10^{4.6}$	$10^{3.6}$	5/5
	$10^{3.6}$	$10^{2.6}$	5/5
WITat 4	$10^{5.3}$	$10^{4.3}$	5/5
	$10^{4.3}$	$10^{3.3}$	5/5
	$10^{3.3}$	$10^{2.3}$	5/5

[1] each mouse received 0.1 ml of suspension

In vivo test

T. brucei brucei (stabilate WIG 76, WITat 1) was retrieved from the cryobank and rapidly thawed at room temperature. Six mice were inoculated intraperitoneally with the diluted stabilate. Infection was confirmed in all the mice by microscopic examination of their tail blood.

All the mice were bled from the orbital plexus and a total of 4 ml of heparinized blood was collected from the mice. Three ducks, aged 1.5 years, were each inoculated intravenously with 1 ml of the parasitaemic blood diluted (1 : 1 with saline), each duck received $10^{7.8}$ mouse infective organisms (ID_{63}). The course of the infection was monitored by a combination of microscopic examination of wet blood preparation and mouse inoculation on days 3, 7, 14, 28, 42, 56, 72, 90 and 120. Control infectivity test was carried out in four mice, each of which received 0.1 ml of blood suspension.

Infections with heterologous antigen types. Three ducks were each inoculated with 3 ml of parasitaemic mouse blood containing $3 \times 10^{7.8}$ trypanosomes of mixed variable antigen types derived from WITat 2, WITat 3 and WITat 4 after each had established infections in mice. Control infectivity test was carried out in four mice.

Diagnosis was carried out on days 7, 14, 21, 28, 42, 56, 72, 90 and 120 by the standard methods of mouse inoculation and microscopic examination of wet blood preparation as previously described by Joshua et al. (1982).

Haemagglutination tests were carried out as described by Parratt and Cobb (1978). Briefly serum samples were obtained from the inoculated ducks on day zero just before the inoculation of trypanosomes and on days 3, 7, 18, 28 and 42 for indirect diagnosis using the heterophile agglutinin response. Serial dilutions of duck serum were incubated at 37°C for 3 h with 2% suspension of washed erythrocytes from rat, rabbit and guinea pigs respectively. Each serum dilution received erythrocytes from only one animal.

Results

Observations from mice inoculated with *Trypanosoma brucei* which were incubated with duck serum showed that duck serum, unlike human serum, is not naturally trypanocidal to *Trypanosoma brucei brucei*. All mice became infected with trypanosomes (Table 1). Most mice that received a low inoculum were infected.

Attempt to induce infection by a homogenous population. Microscopic examination of wet blood preparation and mouse inoculation failed to reveal trypanosomes in the blood of the dukes. This experiment therefore showed that *T. b. brucei*, (WITat 1) is not infective to ducks.

Infections induced by mixed antigen types. Groups of mice inoculated with blood collected on days 3 and 7 exhibited patent parasitaemia (Table 2). None of the mice inoculated with blood collected from the test ducks on days 14, 21, 28, 42, 56, 90 and 120 was found

Table 2

Comparison of infections produced by variable antigen types of *T. brucei* (Groups of 3 ducks

VAT inoculated into ducks	Number inoculated	Duck identity	Results of blood sub-inoculation into mice									
			Days after inoculation									
			3	7	14	21	28	42	56	72	90	120
		A	−	−	−	−	−	−	−	−	−	−
WITat 1	$10^{7.8}$	B	−	−	−	−	−	−	−	−	−	−
		C	−	−	−	−	−	−	−	−	−	−
Mixture of WITat 2 WITat 3	$3\times10^{7.8}$	D	−	+	−	−	−	−	−	ND	−	−
		E	+	+	−	−	−	−	−	ND	−	−
WITat 4		F	+	+	−	−	−	−	−	ND	−	−

+ — at least one of two mice inoculated with duck blood became parasistaemic.
− — All mice remained aparasitaemic, ND — not done.

infected with trypanosomes (Table 2). A transient infection may therefore be said to have been initiated.

Heterophile antibody response induced by mixed antigen types. Reciprocal of serum dilution that agglutinated erythrocytes from different animals is shown in Table 2. Sera from all infected ducks agglutinated erythrocytes from both rabbit and guinea pig. Rat blood cells were not of any diagnostic usefulness since normal duck serum was found to possess a naturally occurring agglutinin against

rat erythrocytes. High agglutinin titre were observed from day 3, reached a peak on day seven and then fell precipitously, on day eighteen and was almost at preinfection level on day 42. The rapid increase in heterophile agglutinin suggests that the trypanosomes were rapidly cleared from the blood of ducks by immune reactions.

Discussion

Although these investigations have been conducted on a limited number of ducks, nevertheless this stock of *T. brucei* could only produce a transient infection in adult ducks. These studies have corroborated the work of Durham (1908) who showed that different stocks of the same species of *Trypanosoma brucei* behaved differently in kesterel and pigeon i.e., different birds. There had been no similar studies on trypanosomes of the same serodeme. On the other hand, Corson (1931) reported that he could infect guinea fowls, domestic fowls and francolin with *Trypanosoma brucei rhodesiense* i.e., his own stock infected various birds tested. Since Corson's work was not carried out with cloned trypanosomes it is difficult to say whether he used the same VAT to infect the birds.

Inoculation of a single antigen type WITat 1 could not infect the ducks, whilst a cocktail of three variable antigen types (WITat 2, WITat 3 and WITat 4) resulted in a transient infection. Infectivity in general depends on a complex set of interactions between the invading organisms and the defense mechanisms of the host. A possible hypothesis for the ability of multiple antigen types to produce a transient infection in ducks could be due to their overwhelming effect on the immune apparatus of the ducks. It is difficult to conclude that these mixed antigen types have produced *Trypanosoma brucei* infection in ducks in spite of demonstration of recovery of mouse infective organisms even on days three and seven post inoculation. Such trypanosomes could be regarded as hardy survivors from the numerous heterotypes present in the mixed inoculum. It is equally important to consider the role of number of organisms in the present investigation. Studies by Bailey and Boreham (1969) showed that 10 mouse infective organisms are required to initiate *T. rhodesiense* infections in man while Joshua (1979) showed that 100 mouse ID_{63} are needed to initiate *T. brucei* infections in domestic chickens. It is therefore likely that the number of infective organisms needed to initiate persistent *T. brucei* infections in ducks is very high.

It has also been shown in these studies that duck serum was not

trypanocidal to *Trypanosoma brucei*. The inability of these organisms to produce a persistent infection cannot be attributed to a naturally occurring antibody present in normal duck serum. The transient nature of the infection might also be attributed to the internal physiological environment of ducks. The normal body temperature of ducks (41.6°C) is higher than that of mammals. It is known that temperature affects the course of diseases in many animals. Type III *Pneumococcus* loses its capsule at the height of fever and becomes susceptible to phagocytosis in rabbits (Mims 1977). On the other hand Woo and Soltys (1969) found that turtles needed to be acclimatized to 37°C to get infected with *T. brucei*. Mathur (1972) reported a spontaneous cure in mice infected with *T. evansi* and maintained at 35°C while controlled mice maintained at 22–26°C developed an acute infection.

During the period that trypanosomes survived in the present ducks, serum agglutinins to erythrocytes from rabbit and guinea pigs were high. It is also clear from this observation (Table 3) that the test of

Table 3

Heterophile agglutinins in duck serum following inoculation of *Trypanosoma brucei* (mixed antigen type)

Days post inoculation	Titres[1]		
	Rat blood cells	Rabbit blood cells	Guinea-pigs blood-cells
0	256	0	0
3	388	388	48
7	2048	512	192
18	4096	128	16
28	2048	32	8
42	256	8	8

[1] Arithmetic mean of reciprocal for serum dilution in three ducks.

heterophile antibodies could be useful in the seroepidemiology of the infection in ducks, since a high titre was recorded weeks post cure of infection. The quick fall in elevated agglutinin titre is confirmation of the observation by White et al. (1970) about the rapid shut-off of antibody response in avian hosts following antigenic stimulation.

Young, week-old, ducklings were infected by Seager (1944) with *T. brucei equiperdum* and some died within four days but others survived with a prolonged latent infection. It is possible that these birds are not immunological competent at the time they were infected. It might be that adult ducks which have well developed immune apparatus might be insusceptible to this stock of *Trypanosoma brucei*.

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