PL ISSN 0065 – 1583

POLISH ACADEMY OF SCIENCES
NENCKI INSTITUTE OF EXPERIMENTAL BIOLOGY

ACTA PROTOZOOLOGICA

VOLUME 24
Number 2

WARSZAWA 1985

POLISH ACADEMY OF SCIENCES
NENCKI INSTITUTE OF EXPERIMENTAL BIOLOGY

ACTA PROTOZOOLOGICA

International Journal of Protozoology

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ACTA PROTOZOOLOGICA appears quarterly. The indexes of the previous volume appear in No. 1 of the next volume.

Indexed in Current Contents and in Protozoological Abstracts.

ACTA PROTOZOOLOGICA
Vol. 24, No. 2, pp. 77-84 (1985)

Cytological Observations on the Ciliate *Coleps hirtus* Nitzsch, 1817: Vegetative Cell, Binary Fission and Conjugation

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Received on 12 June 1984, *revised* 1 October 1984

Synopsis. Some new observations about the general morphology and the infraciliature of the ciliate *Coleps hirtus* are reported. For the first time the composition and arrangement of the brosse is described in this species. The morphogenesis is telokinetal, it begins by a proliferation of the somatic kinetosomes at the equatorial zone. The new brosse in the opisthe is derived by duplication and later fragmentation of the anterior extremes of three somatic kinetics of the parental cell. The conjugation includes three maturation divisions. The synkaryon divides only once, one of its derivates becomes the micronucleus and the other forms the macronuclear anlage. The old macronucleus of each partner degenerates by pycnosis without fragmentation.

Coleps hirtus Nitzsch, 1817 is a gymnostome ciliate belonging to the Order *Prostomatida*, Suborder *Prorodontina* (Levine et al. 1980). Since the revision of the genus *Coleps* made by Noland (1925) and the brief description by Kahl (1930-1935), several ultrastructural studies of this species have been carried out (Fauré-Fremiet et al. 1946, 1968, Grain et al. 1973, Rodrigues de Santa Rosa 1976). The most recent review of the genus *Coleps* has been made by Chardez (1976), this author gave some data about the history of the different species in this genus. Several morphological characteristics and intraspecific variations in the clonal reproduction on *C. hirtus* are also described in this study. However, we still lack a description of the infraciliature or a morphological characterization similar to that made by Wilbert and Schmall (1976) of *Coleps nolandi*.

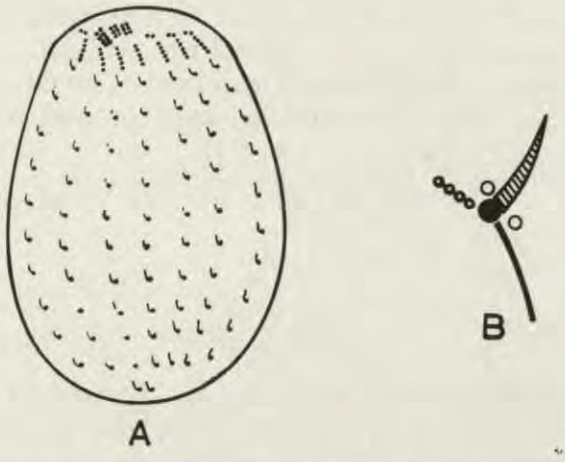

Fig. 1. Semidiagrammatic figure showing: *A* — the infraciliature and the nuclear apparatus of *Coleps hirtus*, and *B* — fibrillar system and parasomal sacs associated to each somatic kinetosome

bears a caudal cilium (Pl. I 3). Each somatic kinetosome presents three types of fibres (kinetodesmal fibre, post-ciliary fibre and transverse fibre) and two parasomal sacs (Fig. 1 *B*, Pl. I 3, 4).

The circumoral infraciliature is made up of two zones: (1) a posterior one with 3-5 kinetosomes closer together than the somatic kinetosomes, and (2) a circumoral kinety made by pairs of kinetosomes. The kinetosomes of these pairs do not have kinetodesmal fibre. The circumoral ciliature is interrupted by three short oblique rows each with 3-4 pairs of kinetosomes. These three rows make up the brosse (Fig. 1 *A*, Pl. I 2).

The silver stain method facilitated an accurate observation of a system of argentophilic lines (Pl. I 5, 6). This system consists of several longitudinal lines parallel to the somatic kinetics and transverse connections between two adjacent longitudinal lines. All seem to be situated in the contact zone of the secondary skeletal plates.

Bipartition

Bipartition is initiated by a proliferation of kinetosomes stretch out from the middle zone of each somatic kinety, and then move towards the anterior and posterior zones of the body (Pl. I 7, 8). In the opisthe, the brosse is formed by first a duplication and then a separation of the anterior ends of three dorsal somatic kineties. These three segments

move into an oblique position with respect to the antero-posterior body axis. The proter conserves the brosse of the parental cell.

During the macronuclear division a chromatin extrusion process was observed which takes place when micronuclear mitosis and the fission of the somatic kineties have started (Pl. I 7).

Conjugation

Conjugant Union

The union between the conjugants has two phases: (1) the cells are first linked by their circumoral cilia (Pl. I 9), and (2) later the union becomes closer so that both buccal overtures come into contact (Pl. II 15). This "closer union" can be observed only during the exchange and fusion of pronuclei. After this fusion, the separation of the partners begins (Pl. II 16).

Feeding in both cells is impossible during the conjugation process.

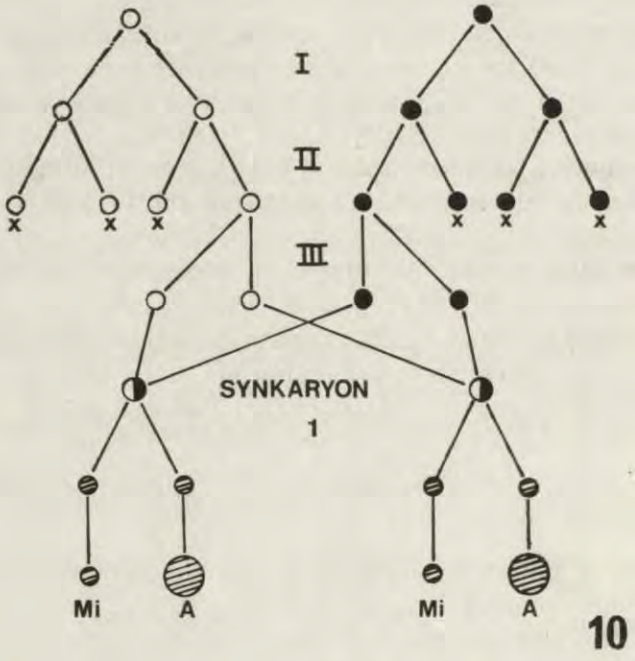

Fig. 10. Diagram of nuclear behaviour during conjugation in *Coleps hirtus*. I, II III — maturation divisions of the micronucleus, 1 — synkaryon division, Mi — micronucleus, A — macronuclear anlage. Crosses mark degenerating nuclei

Nuclear Phenomena (Fig. 10)

First, the micronucleus emigrates from its original location (Pl. II 11) in the vegetative cell to a region closer to the oral area. Then it enters prophase I and becomes voluminous. A characteristic of this phase is the "crescent stage" in which the micronucleus has a ribbon-like shape because the chromosomes follow the line of the "crescent" (Pl. II 12). At the end of two maturation divisions (Pl. II 13, 14) of each micronucleus there are four haploid nuclei in each conjugant (Pl. II 14), of which only the nucleus nearest to the buccal overture divides (third maturation division) (Pl. II 15). The other three nuclei gradually degenerate.

The third maturation division takes place in the union area of both partners. This division gives rise to the migratory and stationary pronuclei without any morphological differences between them (Pl. II 15). The migratory pronucleus of one conjugant moves towards the other mate and fuses with its stationary pronucleus resulting in a voluminous synkaryon (Pl. II 16). From this moment a gradual separation of the pair can be observed, it is finished when the first synkaryon division is over (Pl. II 17).

Each exconjugant presents two diploid nuclei and one part of the old macronucleus. The old macronucleus becomes progressively pycnotic and disappears. After the mates have separated, one nucleus develops into micronucleus and the other into a macronuclear anlage (Pl. II 18, 19).

Discussion

In *Coleps hirtus* the infraciliature is very similar to that described in *Coleps nolandi* (Wilbert and Schmall 1976). It has 13 meridian somatic kineties (*C. hirtus* has 13-14 kineties), the circumoral ciliature consists of two zones and the brosse is made up of three double rows of kinetosomes. However *C. nolandi* is bigger than *C. hirtus* and also the configuration of the windows of the skeletal plates is very different in both species (Kahl 1930-1935, Wilbert and Schmall 1976, Chardez 1976).

Each somatic kinetosome presents three types of fibres: kinetodesmal fibre, transverse fibre and postciliary fibre, as Rodrigues de Santa Rosa (1976) describes in his ultrastructural study of this species. We have also observed two parasomal sacs close to each kinetosome of the somatic kineties that had not been reported previously.

We have not want to set if the brosse in *C. hirtus* has a dorsal or marginal position. According to the disposition of the partners during the conjugation, the brosse in *C. hirtus* would be dorsal, as in *Acropisthium mutabile* (Bohatier and Detcheva 1973). However, Fryd-Versavel et al. (1976) consider that in the ciliates with apical and rounded mouth, the brosse has a marginal position because it is not possible to know if the plane of bilateral symmetry defined by this structure corresponds with that defined by the mouth.

The system of argentophilic lines of *C. hirtus* is probably the argyrome of this ciliate already described by Klein (1926), but it does not correspond to the fibrillar tracts found in this species (Rodrigues de Santa Rosa 1976) since these tracts were located along the right side of the somatic kineties and do not have any transversal connections.

The morphogenesis in *Coleps hirtus* seems to be very similar to that reported in other species of the same order (*Enchelys simplex* Dragesco, 1966, *Didinium nasutum* Small et al. 1972, *Fuscheria terricola* and *Spathidium muscorum* Berger et al. 1983, *Acropisthium mutabile* Serrano et al. 1984). The brosse results from a process similar to that described in *Prorodon palustris* (de Puytorac and Savoie 1968). In both species the brosse appears due to the separation of the anterior ends of several somatic kineties, although in *P. palustris* the bipartition takes place within a cyst.

With regard to the conjugation process, the nuclear changes include more or less the same mechanisms as in *Prorodon griseus* (Tannreuther 1926), a member of the same suborder. There are three maturation divisions and only one postzygotic division; however, in our species there are not any significant morphological differences between stationary and migratory pronuclei. The exchange of pronuclei in *P. griseus* (Tannreuther 1926) and *Chilodonella uncinata* (Enriques 1907, MacDougall 1925, 1936) is also similar. In the three species the third maturation division takes place at the union area of both partners, and both the stationary and the migratory pronuclei are connected by the mitotic spindles (Raikov 1972).

It must be pointed out that in the meiotic prophase I the "crescent stage" and not the "parachute stage" was observed. In the gymnostomes *Didinium nasutum* (Prandtl 1906) and *Urotricha* sp. (personal observations) there has been observed the "parachute stage". Although few studies have been made on the conjugation process in the primitive ciliates, it might indicate that the gymnostomes do not exhibit a common stage ("parachute stage" or "crescent stage") in the prophase I of the first maturation division.

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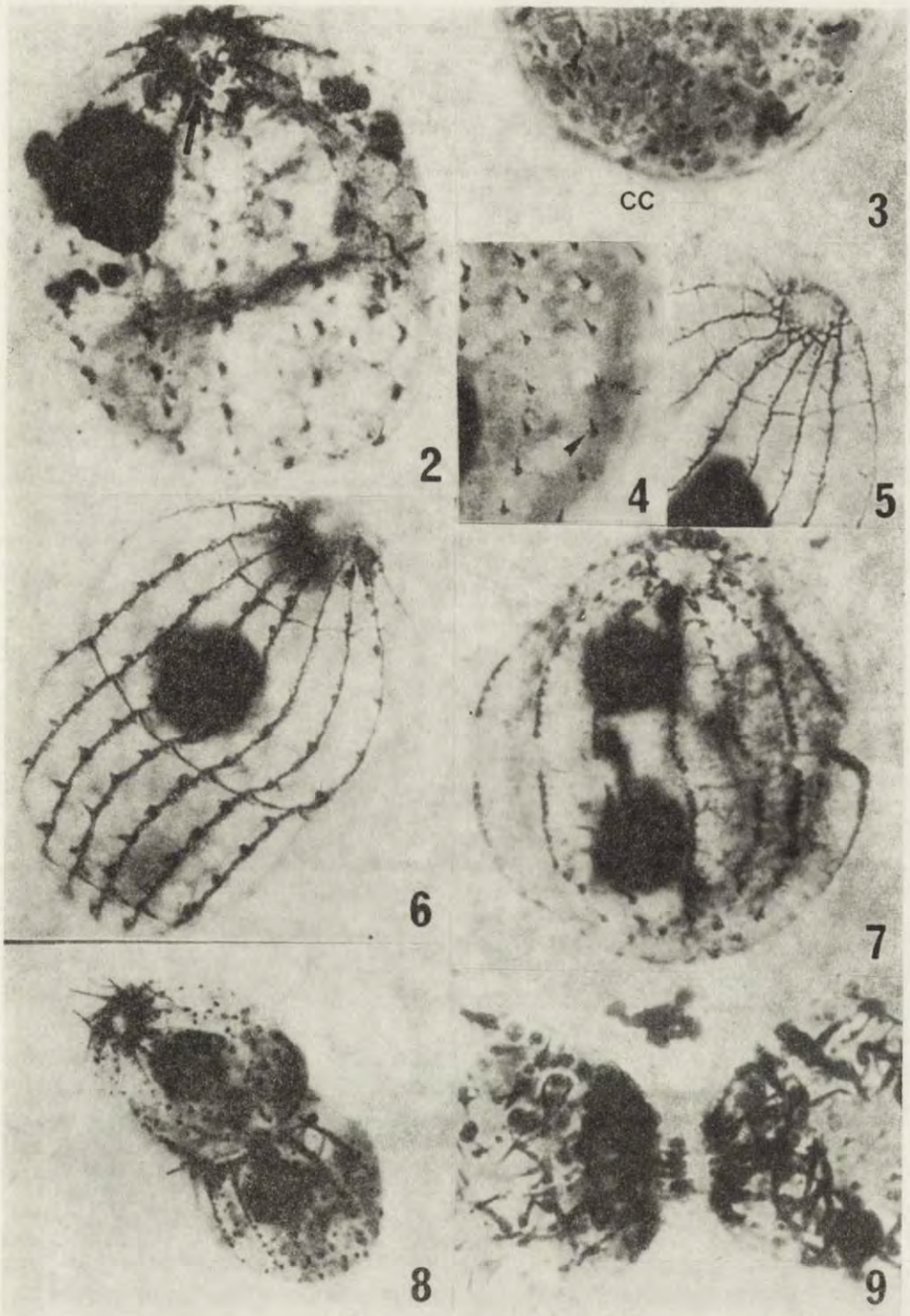
EXPLANATION OF PLATES I-II

Plate I

2: View of a vegetative specimen. Arrow points to the brosse made of three rows of paired basal bodies. (×3350)
3: Detail of the posterior end of the body, showing the caudal cilium (CC). The arrow indicates the situation of one of the two parasomal sacs placed near to each somatic kinetosome (×4000)
4: Detail of the somatic infraciliature and the fibrillar system associated to each kinetosome. Another parasomal sac placed near to each somatic kinetosome can be seen (arrow). (×3500)
5-6: Photomicrographs of the silverline system in *Coleps hirtus* 5 — Detail of the anterior pole. (×3500). 6 — (×3250).
7-8: Two different stages of the bipartition in Coleps hirtus (for details, see the text). 7 — (×3500). 8 — (×2200)
9: Union of the conjugants in *Coleps hirtus*. In this stage both mates are united by their circumoral cilia. (×4000)

Plate II

11: Beginning of the conjugation process. The division of the micronucleus has not started. (×1400)
12: "Crescent stage" of the meiotic prophase I. (×1400)
13: First maturation division. (×1400)
14: End of the second maturation division. (×1400)
15: A third maturation division takes place in the union area (×2750)
16: After exchange of migratory pronuclei, a synkaryon (S) is formed in each conjugant. (×1400)
17-19: Exconjugants of *Coleps hirtus*. The most posterior derivate from the synkaryon division develops in macronuclear anlage (A). (×2200)



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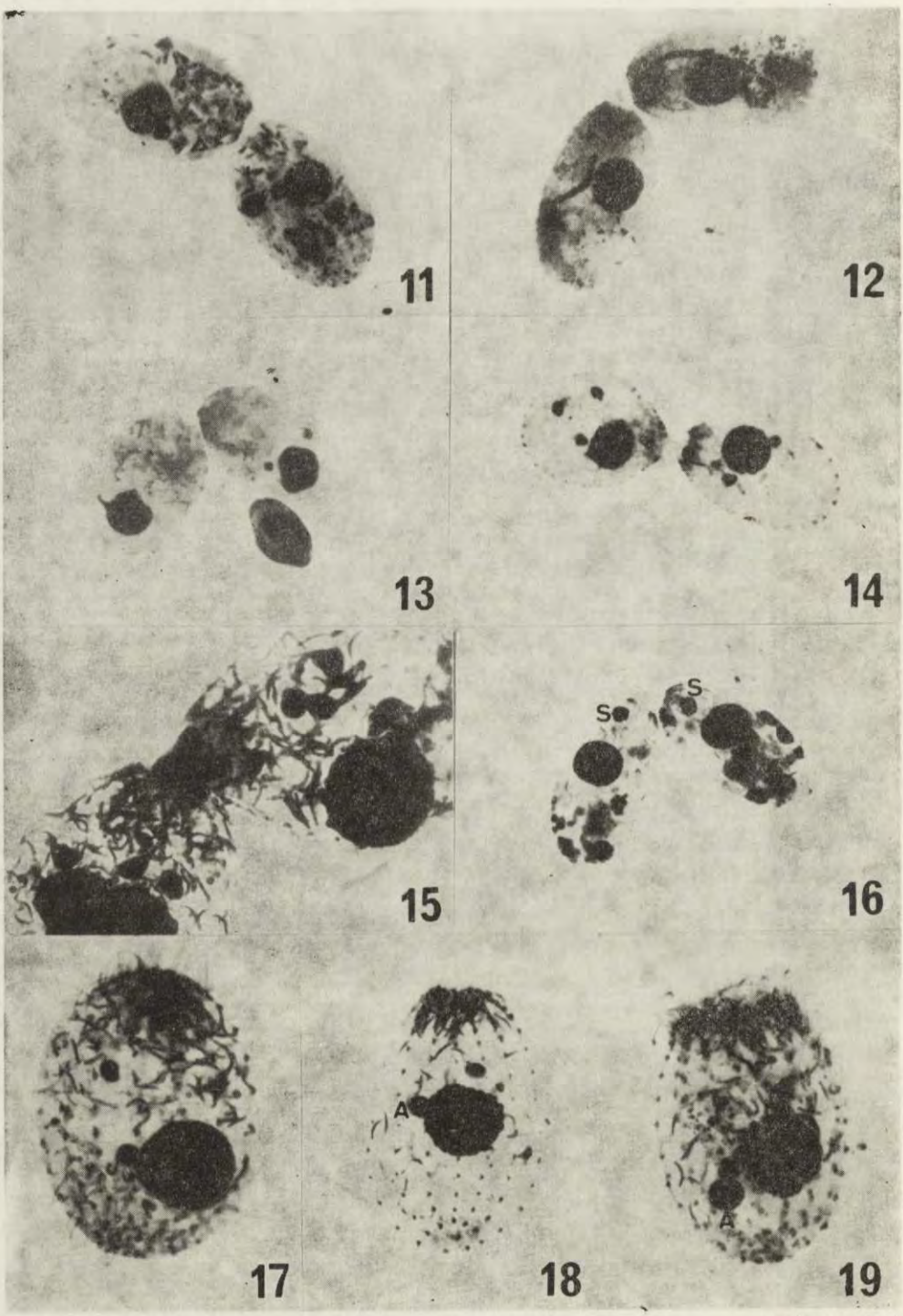

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ACTA PROTOZOOLOGICA
Vol. 24, No. 2, pp. 85-109 (1985)

The Effects of Lead on some Protozoan Communities. Ciliates of the Santillana Reservoir and Ciudad Universitaria (Madrid, Spain)

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Received on 27 March 1984, *revised on* 17 July 1984

Synopsis. Studies have been carried out on the effects of different lead concentrations over the media commonly found in polluted areas, employing freshwater samples from the Santillana reservoir and from the Ciudad Universitaria of Madrid. Ciliate protozoan communities, the variation of ecological conditions, and lead accumulation by the ciliates have been analyzed. Several species of this type of organisms, likely to be used as pollution indicators, have been found.

The excess of heavy metals introduced into marine ecosystems is owed to products of industrial origin and acid mining drainage residues. Investigations on the effects of certain materials, for example Zn, Cu, Cd, Li, Cr, Hg, Ni and Pb, on seaweeds and protozoa are under an increasing trend.

Studies on the biological treatment of running water indicate that normal purification decreases or is actually checked owing to heavy metals (specially so with Cu and Pb), and that protozoan development is slowed down by copper concentrations above 0.5 mg/l. Some metals (including copper), on a 1-10 mg/l concentration, during a prolonged period, cause a reduction of activated sludge efficiency.

As to lead, it is generally known to be amongst the more abundant metals in nature, and its use by man dates far back into civilizations and cultures. At present, industrial processes employing lead are numerous.

Lead is amongst the different metallic elements that show highest dispersion, and therefore it reaches far out from its primary source, which explains its very high contaminating dispersion power. Environmental lead originates from natural sources and anthropogenic sources. The highest environmental pollution level through lead has anthropogenic origin. There are two clearly defined origins for this contamination:

(1) Industrial origin,

(2) Vehicle gas combustion origin.

The principal contamination source of anthropogenic origin is found in fuel combustion with an anti-knocking compound, which in some parts is practically responsible for 90% or more of existing environmental lead.

It has been shown that there exist seasonal variations of the average lead concentration in the atmosphere. Furthermore, airborn lead from its original source has a complex network of inroads interconnected to the biosphere (hydrosphere, atmosphere and lithosphere).

Air lead diffusion is influenced by the intensity of the wind, its speed and turbulence intensity, which can be favourable or not to its propagation. Moreover, particle size and physical characteristics of lead are determinating.

Particles constitute the fundamental source for lead outcome. Prevailing sizes of outcoming particles are: small particles (under 1 μm), medium particles (between 5 and 50 μm), and larger particles between 300 and 3000 μm in diameter). Lead present in the atmosphere is eliminated by rotary action and wasted away by rain. It is transferred from the air to the earth and rivers. Once emitted, the large lead particles fall to the ground near the source of emission, while particles under 10 μm diameter may remain suspended a long time in the atmosphere and circulate from 7 to 30 days approximately. It is believed that there exists a great proportion of under 1 μm diameter particles in the atmospere (approximately 60-70%).

Lead, on the other hand, is to be found under higher concentration in city soils rather than rural ones, lead polluted dust on the soil is an important contaminating source and in urban areas is responsible for lead in the air.

On the other hand, suspended particles in the atmosphere, owing to their small size, may travel long distances from their source; thus, for example, there is information concerning the presence of atmospheric lead in zones far away from civilization. In Greenland, lead concentration reaches 0.0001-0.001 $\mu g/m^3$.

It has also been observed that there exists a relationship between tissue lead concentration and particle size. Lead, moreover, accumulates in the tissues because its absorption/excretion balance is positive, that is to say, in favour of absorption.

Materials and Methods

The ciliate protozoan communities come from samples obtained from the Santillana reservoir and the Ciudad Universitaria (Madrid, Spain). Collected samples were enriched with wheat grains under sterile conditions (one wheat grain for each 50 ml sample).

Samples holding a more numerous ciliate community which developed were treated with lead acetate. One of the Santillana samples (A) was divided into five fractions and placed in different containers (1A, 2A, 3A, 4A, 5A). One fraction (1A) was kept as control and the other four (2A, 3A, 4A, 5A) were treated with increased lead acetate concentrations, from 5 μg/l to 1000 μg/l, corresponding to the minimum and the maximum concentrations found by various authors in the polluted waters. Samples from the Santillana reservoir were treated with lead acetate 6 days after they were collected. Thereafter, the samples underwent examination for a month. Fraction 2A was treated with 5 μg/l of lead acetate, 3A with 100 μg/l, 4A with 500 μg/l and 5A with 1000 μg/l. Another Santillana sample (B) was divided into two fractions, one (1B) was kept as reference control and the other (2B) was treated with 500 μg/l of lead acetate.

Samples from the Ciudad Universitaria were treated with lead acetate the day after they were collected. Thereafter, the samples underwent examination for a month. One of the Ciudad Universitaria samples (C) was divided into 5 fractions and placed in different containers (1C, 2C, 3C, 4C, 5C). One of the fractions (1C) served as reference control, the other fractions (2C, 3C, 4C, 5C) were treated with increased lead acetate concentrations, to fraction 2C were added 5 μg/l, to fraction 3C, 100 μg/l, to fraction 4C, 500 μg/l, and to fraction 5C, 1000 μg/l of lead acetate. Another sample from Ciudad Universitaria (D) was divided into two fractions; fraction 1D served as control and to 2D were added 500 μg/l of lead acetate.

The ciliate communities in the samples were first observed with the help of a stereomicroscope and the abundance of each species was calculated for a 10 ml sample, directly when possible. When the number of specimens became too high, calculations were carried out by dilutions.

To identify the different ciliate species, specimens were examined and determined, in some cases with a ligth microscope, and in others it became necessary to use vital staining. Specific techniques, such as pyridinated ammonical silver carbonate (Fernandez-Galiano 1976) or the Protargol technique (Tuffrau 1967) were applied to some ciliate species of more problematic classification, as well as to the study of some cytological pecularities, as infraciliature for example (Corliss 1979, Kahl 1935).

To reveal lead deposits in the cells, three histochemical techniques were

used: Rhodizonic acid sodium salt, Potassium dichromate and Hematoxyline-Eosine, the first two are specific for lead (Humason 1979).

From the day the samples were obtained and during a month, at 48 h intervals, the following factors were examined: pH, dissolved oxygen, and temperature (Rodier 1978).

Results

The Ciliate Communities

Sample collection at the Santillana reservoir was carried out on May 24th. The same day, there were found small numbers of *Prorodon ovum* (2 specimens/10 ml), *Colpidium colpoda* (10 specimens/10 ml), *Stylonychia mytilus* (2 specimens/10 ml), *Vorticella microstoma* (1 specimen/10 ml) and *Frontonia leucas* (1 specimen/10 ml) (Fig. 8).

After 48 h, the community had undergone a slight modification: *Vorticella microstoma* increased to 15 specimens/10 ml, and three species appeared in addition: *Litonotus lamella* (2 specimens/10 ml), *Tachysoma pellionella* (2 specimens/10 ml) and *Amphileptus claparedei* (2 specimens/10 ml). On the sixth day after the samples were collected, and previously to the treatment with lead acetate, the ciliate communities underwent a further modification (Fig. 8): *Keronopsis rubra* (4 specimens/10 ml), *Histriculus similis* (1 specimen/10 ml), *Stentor roeseli* (8 specimens/10 ml) and *Paramecium bursaria* (2 specimens/10 ml) appeared, *Vorticella microstoma* increased to 20 specimens/10 ml. However, *Prorodon ovum*, *Amphileptus claparedei*, *Litonotus lamella*, *Frontonia leucas*, *Colpidium colpoda*, *Tachysoma pellionella*, and *Stylonychia mytilus* disappeared.

Sample collection at the Ciudad Universitaria was carried out on May 24th. The ciliate community found in this zone basically consisted of *Paramecium caudatum* and *Paramecium bursaria* (2 specimens/10 ml and 4 specimens/10 ml respectively), *Lembadion* sp. (5 specimens/10 ml), *Stylonychia mytilus* (2 specimens/10 ml) and *Euplotes eurystomus* (4 specimens/10 ml). The community of this composition remained until it was treated with lead acetate (Fig. 9).

Physico-Chemical Factors

Oxygen (Tables 1 and 2). As to dissolved oxygen in samples from the Santillana reservoir, the fraction 1A used as a general control sample showed an increase in oxygen concentration from 8.40 mg/l to 9.50 mg/l. Only on the 17 day there was a small drop in oxygen

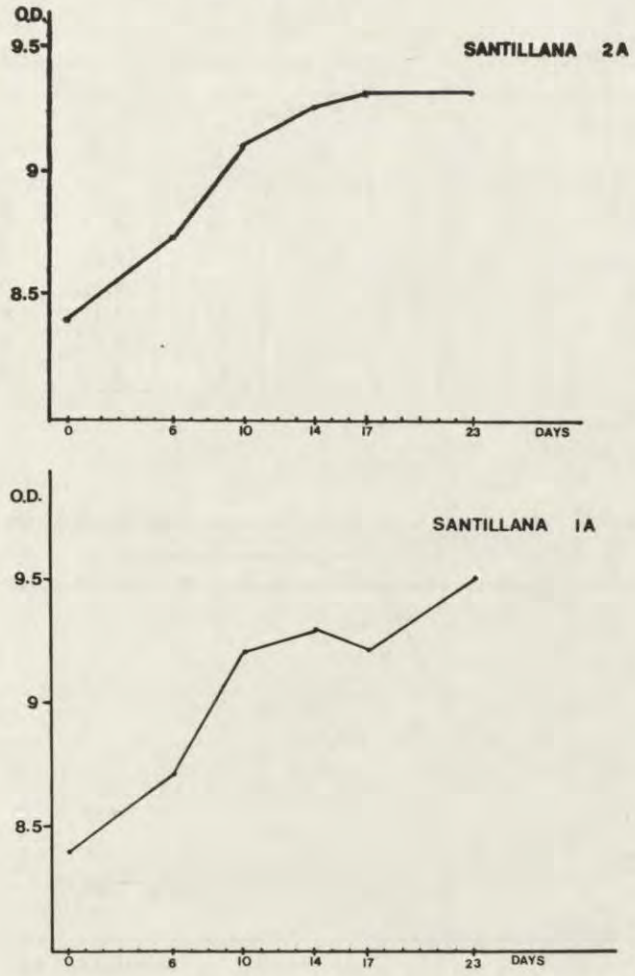

Fig. 1. Variation in oxygen concentration in fractions 1A and 2A, Santillana samples

concentration to 9.20 mg/l (Fig. 1, bottom). In fraction 2A, to which 5 μg/l lead acetate was added, no oxygen concentration drop was observed on the 17th day, and from then to the 23th day the oxygen concentration remained constant at 9.30 mg/l (Fig. 1, top). To fraction 3A, 100 μg/l of lead acetate was added and the curve line was very similar to that of control sample, with the same oxygen concentration drop on the 17th day (9.20 mg/l) and an equal value by the end of the measurement period (9.50 mg/l) (Table 1). In the 4A fraction, to which 500 μg/l lead acetate was added, the drop on the 17th day was not observed, and the oxygen concentration reached the control level

Table 1

Dissolved oxygen in the samples from Santillana reservoir (mg/l)

	(days)					
	0	6	10	14	17	23
1A	8.4	8.72	9.2	9.29	9.2	9.5
2A	8.4	8.72	9.1	9.27	9.3	9.3
3A	8.4	8.72	9.1	9.3	9.2	9.5
4A	8.4	8.72	9.2	9.27	9.28	9.5
5A	8.4	8.72	9.1	9.3	9.3	9.5
1B	8.4	8.72	9.2	9.29	9.2	9.5
2B	8.4	8.72	9.2	9.3	9.3	9.3

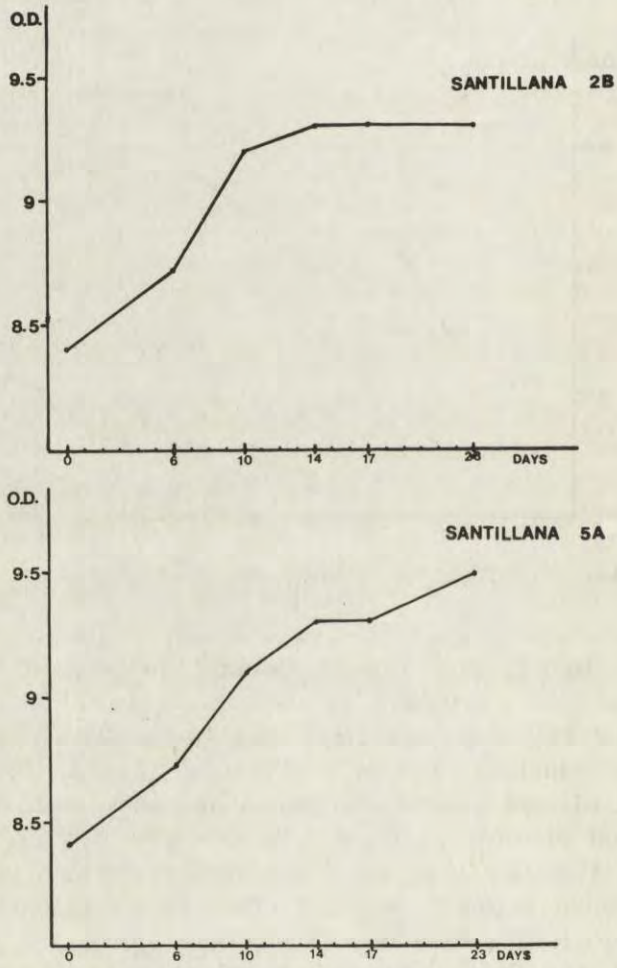

Fig. 2. Variation in oxygen concentration in fractions 5A and 2B, Santillana samples

on day 23 (Table 1). In fraction 5A (1000 µg/l of lead acetate), the oxygen concentration was unaltered between the 14th and the 17th day (9.30 mg/l), and increased to 9.50 mg/l on day 23 (Fig. 2, bottom).

As to sample B, the control fraction 1B, to which no lead acetate was added, showed very much the same values as those obtained in the control (1A) of sample A (Table 1). On the other hand, in fraction 2B, containing 500 µg/l of lead acetate, the oxygen concentration variation differed from the control sample: no 17th day concentration drop and no increase from the 17th to the 23th day was observed (Fig. 2, top).

In Ciudad Universitaria samples, the sample C presented in the

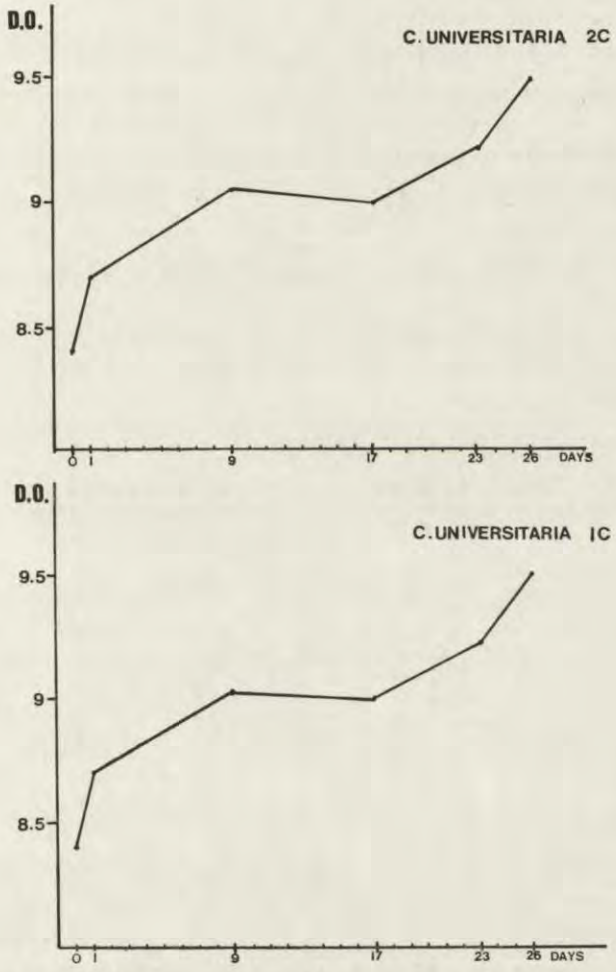

Fig. 3. Variation in oxygen concentration in fractions 1C and 2C, Ciudad Universitaria samples

Table 2

Dissolved oxygen in the samples from Ciudad Universitaria (mg/l)

	(days)					
	0	1	9	17	23	26
1C	8.4	8.73	9.01	9.0	9.22	9.5
2C	8.4	8.76	9.06	9.0	9.22	9.5
3C	8.4	8.70	8.80	9.0	9.22	9.5
4C	8.4	8.75	9.01	9.0	9.22	9.5
5C	8.4	8.78	9.06	9.0	9.22	9.5
1D	8.4	8.73	9.01	9.0	9.22	9.5
2D	8.4	8.74	8.74	9.2	9.22	9.5

Table 3

pH of the samples from Santillana reservoir

	(days)					
	0	6	10	14	17	23
1A	10.23	9.35	6.36	6.53	6.59	7.09
2A	10.23	9.35	6.80	7.62	7.39	7.09
3A	10.23	9.35	6.992	7.12	7.31	6.92
4A	10.23	9.35	6.990	7.10	7.19	7.19
5A	10.23	9.35	6.991	7.04	6.71	7.15
1B	10.23	9.35	6.36	6.53	6.59	7.09
2B	10.10	7.71	6.79	6.78	6.767	6.69

Table 4

pH of the samples from Ciudad Universitaria

	(days)					
	0	1	9	17	23	26
1C	6.79	8.73	8.77	8.53	7.63	8.29
2C	6.79	8.71	8.71	8.53	7.63	8.29
3C	6.79	8.73	8.80	8.53	7.63	8.29
4C	6.79	8.68	8.74	8.53	7.63	8.29
5C	6.79	8.71	8.77	8.53	7.63	8.29
1D	6.79	8.73	8.77	8.53	7.63	8.29
2D	7.62	8.24	8.46	8.63	8.46	8.81

control (1C) a minimum oxygen concentration on day 0 (8.4 mg/l) and a maximum concentration on day 26 with 9.50 mg/l, with just a slight drop corresponding to day 17 (9.0 mg/l) (Fig. 3, bottom). Similar graphs have been obtained in fractions with lead acetate added: 2C (Fig. 3,

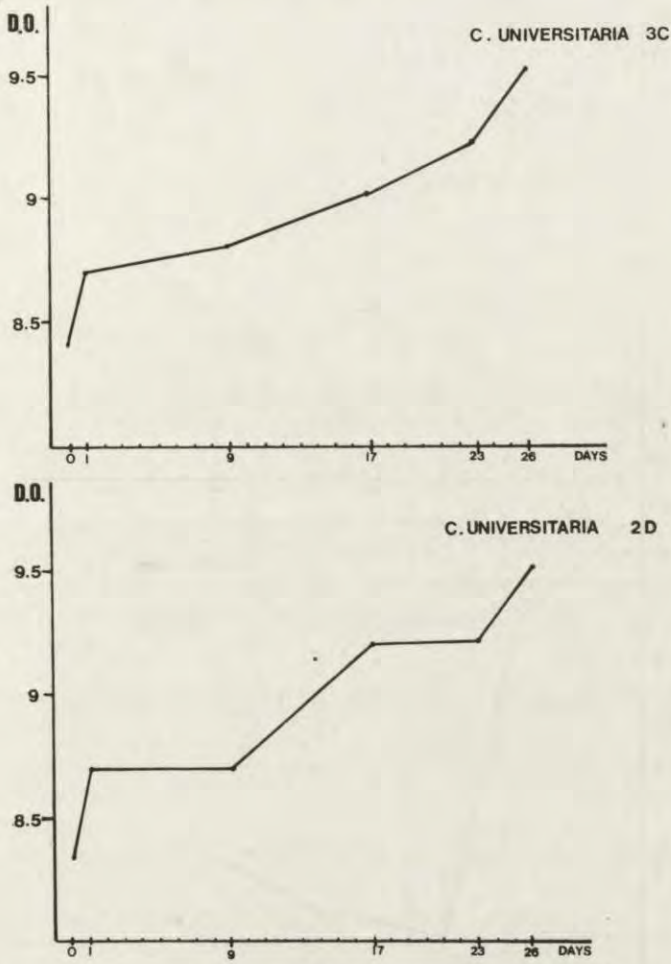

Fig. 4. Variation in oxygen concentration in fractions 3C and 2D, Ciudad Universitaria samples

top), 4C and 5C (Table 2). In fraction 3C (Fig. 4, top), the oxygen concentration increased from day 0 to day 26, with no intermediate minimum.

In sample D, the control 1D showed the same values as in the control sample C (Table 2). In fraction 2D (with 500 μg/l of lead acetate) the oxygen concentration increased within the same limits, but rather stepwise (Fig. 4, bottom).

pH (Tables 3 and 4). In sample A, from the Santillana reservoir, the control fraction 1A showed in general a pH drop from day 0 to day 10 (from 10.23 to 6.36). From day 10, the pH slightly increased to day 23 when it reached 7.09 (Fig. 5, bottom).

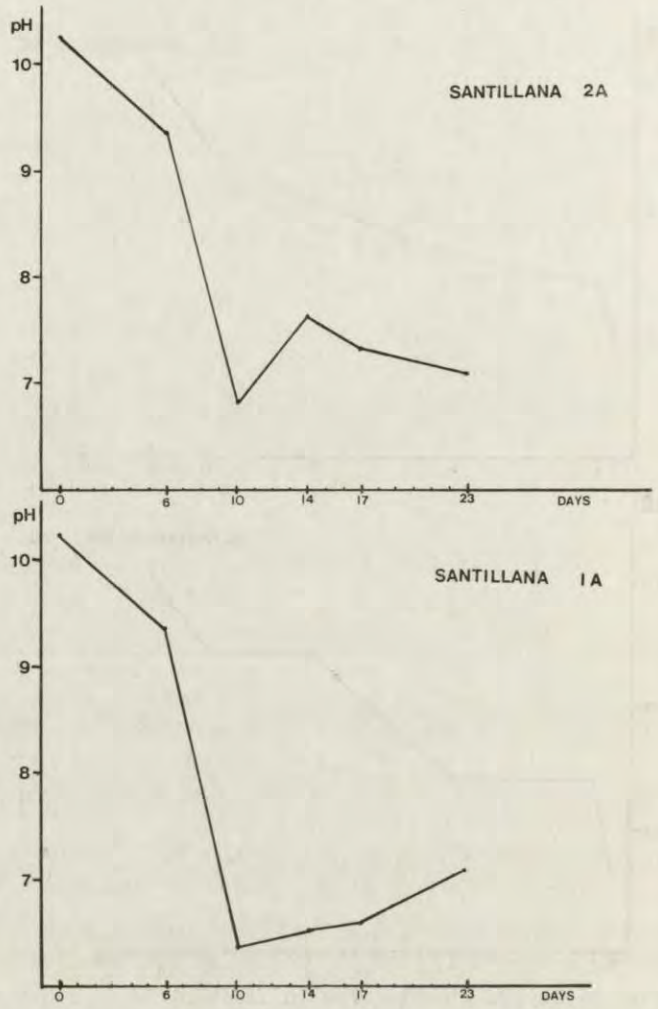

Fig. 5. pH variation in fractions 1A and 2A, Santillana samples

In sample 2A (with 5 µg/l lead acetate), the curve resembled that of the control up to the 10th day, nevertheless, when lead acetate was added, the drop in pH values reached only 6.80 on day 10, while in the control sample 1A it reached 6.36. From day 10 onwards, there was an important increase of pH values on day 14 (7.62 against 6.53 in the control). From day 14, the pH values dropped down to a value similar to that of control sample (Fig. 5, top).

In sample 3A (100 µg/l of lead acetate) the drop of pH from day 6, when lead was added, was much less, and on day 10, values very

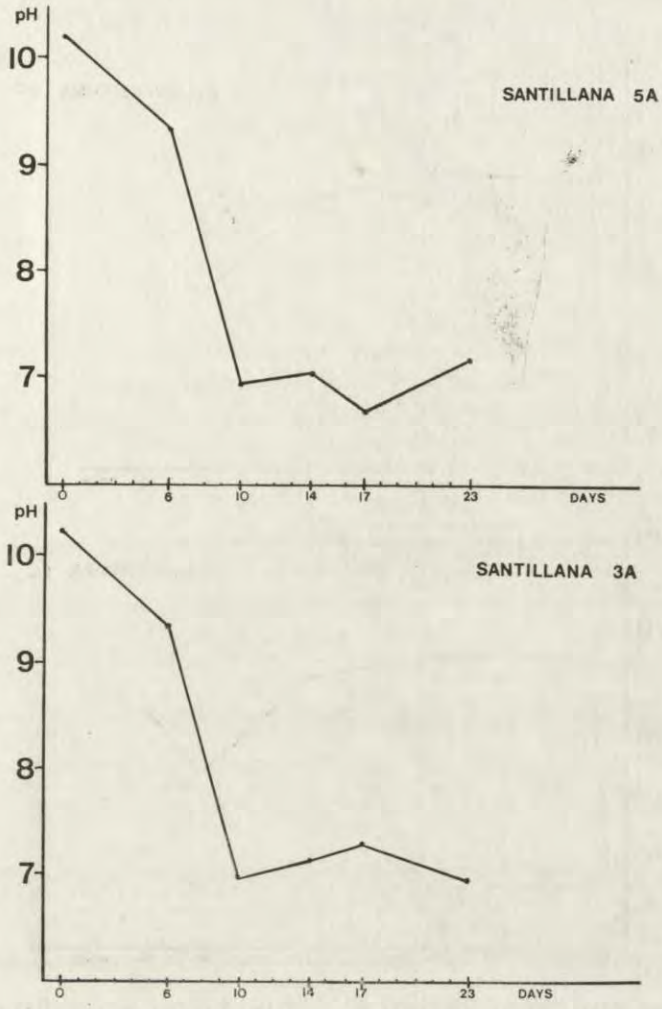

Fig. 6. pH variation in fractions 3A and 5A, Santillana samples

close to neutral pH were observed (6.99). From this day on, an increase in pH occurred, reaching a peak on day 17 (7.31), followed by a decrease to 6.92 on day 23 (Fig. 6, bottom). In fraction 4A (500 μg/l of lead acetate), the pH values were similar to fraction 3A up to day 10 and then increased till day 17, but less than in fraction 3A (Table 3). From this day, contrary to what occurs in other fractions, the pH value remained unaltered (7.19).

In fraction 5A (with 1000 μg/l of lead acetate) the graph up to day 10 was similar to that obtained in fractions 3A and 4A. On day 14,

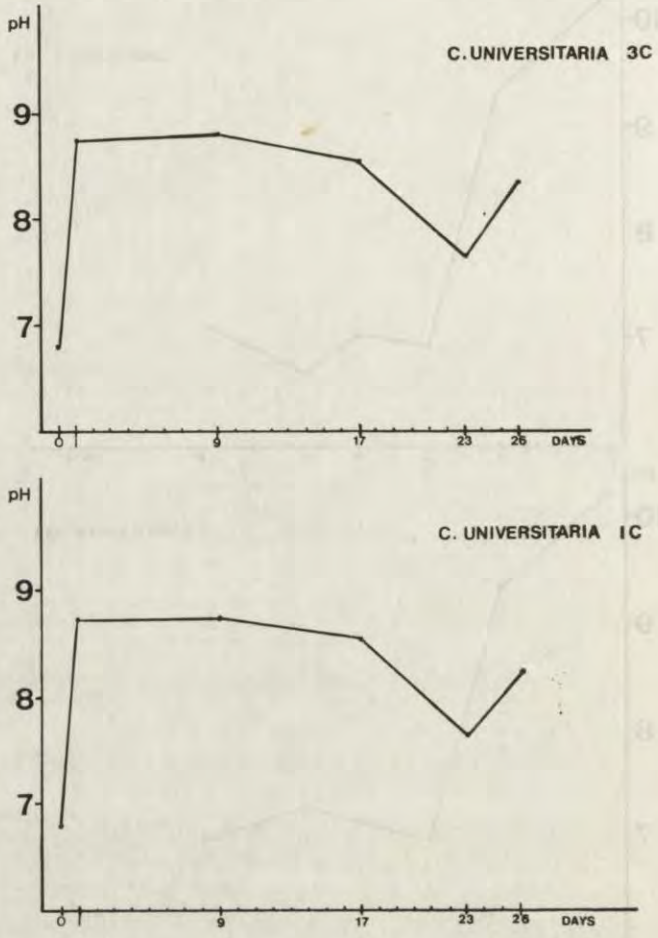

Fig. 7 A. pH variation in fractions 3C and 1C, Ciudad Universitaria samples

a pH 7.04 was reached, and from that moment, the pH at first decreased to 6.71, and then re-increased to 7.15 (Fig. 6, top).

The control sample 1B presented the same variations in pH values as the control of sample A (Table 3). In sample 2B (with 500 µg/l of lead acetate), there was a drop in pH value up to day 10 when a 6.79 value was reached. From that day and up to day 23, the pH variation was only slight (Table 3).

In the sample C, from Ciudad Universitaria, the control 1C presented on day 0 its lowest pH value (6.79), and from day 0 to day 1 this value sharply increased to 8.73. From day 1 to day 17, the pH variation was relatively unimportant, and from day 17 to day 23 the

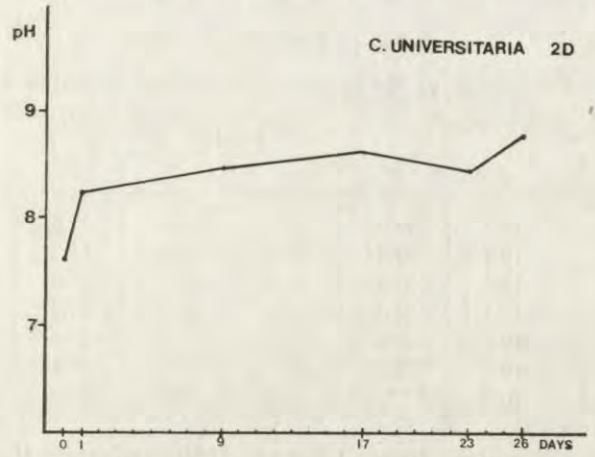

Fig. 7 B. pH variation in fraction 2D, Ciudad Universitaria samples

pH value decreased to 7.63. From day 23 to day 26, the pH again increased to 8.29 (Fig. 7 A, bottom).

Other fractions of sample C showed similar pH graphs to that of the control (Table 4, Fig. 7 A, top). However, in fraction 2D, with 500 µg/l of lead acetate, the pH variation of day 17 to day 23 was considerably less than in sample C fractions, reaching 8.46 on day 23, and 8.81 on day 26 (Fig. 7 B).

Temperature (Tables 5 and 6). The temperatures recorded in the various samples from Santillana reservoir are shown in Table 5. In general, the temperatures decreased from day 0 to day 14, increased on day 17, and decreased again to day 23. This may account for a temporary decrease of oxygen content in some samples about day 17.

Table 5

Temperature of the samples from Santillana reservoir

	(days)					
	0	6	10	14	17	23
1A	26,3	25,6	24.4	24.3	24.9	22.9
2A	26.3	25.6	24.4	23.4	25.4	23.1
3A	26.3	25.6	24.5	23.3	25.4	22.9
4A	26.3	25.6	24.4	23.4	25.6	23.0
5A	26.3	25.6	24.3	23.6	25.6	23.2
1B	26.3	25.6	24.4	24.3	24.9	22.9
2B	26.1	25.8	24.4	24.1	25.2	22.9

Table 6

Temperature of the samples from Ciudad Universitaria

	(days)					
	0	1	9	17	23	26
1C	19.7	25.2	24.9	23.5	25.8	23.8
2C	19.7	25.1	25.0	23.5	25.8	23.8
3C	19.7	25.4	25.4	23.5	25.8	23.8
4C	19.7	25.1	25.3	23.5	25.8	23.8
5C	19.7	25.2	25.0	23.5	25.8	23.8
1D	19.7	25.2	24.9	23.5	25.8	23.8
2D	20.3	25.2	25.2	23.9	25.7	23.8

As to the samples from Ciudad Universitaria, the temperature strongly increased in them when they were brought to the laboratory (from about 20° on day 0 to about 25° on day 1). The further changes are shown in Table 6.

Effects of Lead on the Ciliates

(1) Samples from Santillana Reservoir

The earliest effects were manifested after 4 days of lead treatment, i.e., on day 10. The fractions of sample A, to which 5 μg/l (2A), 500 μg/l (4A) and 1000 μg/l (5A) of lead acetate were added, showed no signs of vegetative forms (Fig. 8). On the other hand, the fraction 3A of the sample, to which 100 μg/l of lead acetate was added, contained *Paramecium bursaria*. It was treated with histochemical techniques. Specimens of *Paramecium bursaria* containing lead deposits were observed; they were easily stained with Rhodizonic acid sodium salt. The lead deposits measured 0.3 μm in diameter (Fig. 10).

In the fraction of sample B to which 500 μg/l of lead acetate was added, *Paramecium bursaria* specimens were also observed, practically all of them had a single deposit of a relatively large size (6 μm) in the preoral suture area. In the same fraction, 2B, specimens of the hypotrich *Histriculus similis* were present, which carried no lead deposits in preparations stained with Rhodizonic acid sodium salt.

The following noteworthy effects were observed 8 days after the treatment began (day 14, Fig. 8). In sample A, fractions with 5 and 1000 μg/l of lead acetate (2A and 5A) had no signs of vegetative forms, while fraction 3A and 4A (with 100 and 500 μg/l of lead acetate) showed some ciliates. In fraction 3A there appeared several specimens of *Stentor roeseli*, showing lead accumulation in form of small non bunching particles (0.6 μm for the particles, and 1.20 μm for the groups) in

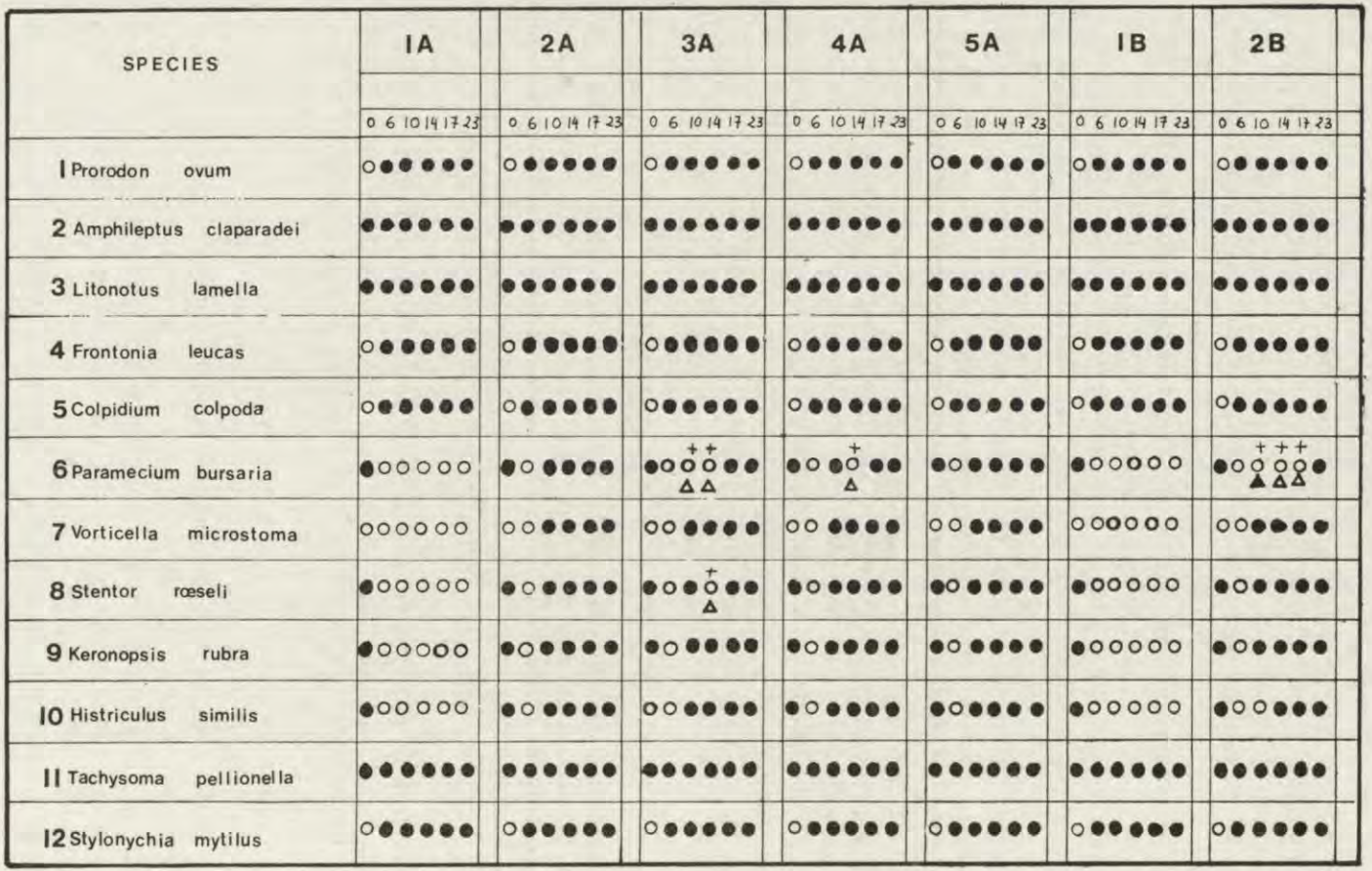

Fig. 8. Lead distribution in the different ciliate species, Santillana samples: + with lead deposits, — without lead deposits, filled triangle — bunching particles, triangle — non bunching particles, filled dot — species absent, dot — species present

the left anterior dorsal zone of the ciliate, near the area of the adoral membranellar zone and the internal rear extreme of the mouth funnel. Other 0.60 and 1.20 μm lead particles were observed in the rear body region of the ciliate, between the foot and the ramification of the principal myonemes. In the some fraction 3A, there were also some specimens of *Paramecium bursaria* containing one to four lead deposits, of which the larger-sized were the more frequent (1.50, 2,25 and 3 μm). In the fractions 4A and 2B, containing 500 μg/l of lead acetate, several specimens of *Paramecium bursaria* were observed, these specimens showed a relatively varied lead distribution, although, in general, a thick lead deposit by the left rear zone of the macronucleus and a variable number (between 1 and 7) of smaller-size particles (0.75 μm) were observed.

On the eleventh day of treatment (day 17), the fractions 2A, 3A, 4A and 5A contained no ciliates. In fraction 2B, a very few specimens of *Paramecium bursaria* were observed, in which eventually some 3 μm lead deposits appeared. From the 17th day of treatment (day 23 of experiment) no ciliate protozoans could be observed in any of the fractions other than the controls (Fig. 8).

(2) Samples from Ciudad Universitaria

The samples treated with lead acetate were studied on the day after treatment. In fraction 2D, with 500 μg/l of lead acetate, used for preliminary observation of sample D, there were specimens of the hypotrich *Stylonychia mytilus*, in which no lead particles appeared.

After 9 days of treatment, the community of *Stylonychia mytilus* became substituted by a community of the hypotrich *Oxytricha fallax* in the same fraction 2D, this new species also showed no lead deposits in its specimens. In a similar way the different fractions of sample C were examined, and no lead deposition was found in the different ciliates under analysis.

After 17 days of treatment, the following samples were examined. In sample C, fraction 2C, to which 5 μg/l of lead acetate was added, contained specimens of *Paramecium caudatum* with 1 or 2 lead bunches of a relatively large size (1.20 μm to 1.50 μm). Generally, one of these bunches is placed near the macronucleus and is the more often observed, while the other, seen only in some specimens, is situated in the ciliate's rear zone. In this fraction, specimens of the hypotrich *Euplotes eurystomus* have also been observed, with relatively large quantities of non-bunching lead particles (between 25 and 36 particles in most of the specimens; each particle hat an average size of 3 μm).

In fraction 3C, to which 100 μg/l of lead acetate was added, several specimens of *Euplotes eurystomus* occurred on day 17. Here also, al-

most all specimens contained non-bunching of lead particles dispersed all over the cell, but specially concentrated in the ciliate's equatorial area and rear part, above the anterior bend of the macronucleus, and in the region adjacent to the left anterior part of the macronucleus. These minute particles, 60 to 100 in number, had approximately the sizes of 3 μm, 0.90 μm and 1.20 μm. In fraction 4C, containing 500 μg/l of lead acetate, specimens of *Euplotes eurystomus* were also seen after 17 days. In these, the lead particles preferably accumulated in the rear half on the individual. The particles, 30 to 40 in number in most of the specimens, were relatively large (6 μm) in comparison with the particles seen in the same species in fraction 3A.

In fraction 5C, with 1000 μg/l of lead acetate, specimens of *Euplotes eurystomus* were found on day 17 in which the lead particles occurred in large quantities in the rear two third of the body. The lead particles were 60 to 100 in number in almost all specimens and they varied in size from 0.3 μm to 6 μm (Fig. 11).

In sample D, fraction 2 D, to which 500 μg/l of lead acetate was added, the lead deposits were present in three species on day 17 (Fig. 9). *Stylonychia mytilus* had generally rounded, relatively large lead particles, 1.20 and 1.80 μm in size, which were present in small number (between 5 and and 11), and were located preferably in the zone near the adoral membranelles. In the same fraction, specimens of *Histriculus similis* have been observed, in which lead was found in more or less spheric non-bunching particles in the rear half of the individual. These particles occur in relatively small numbers (between 4 and 8) and have comparatively large diametres (1.20 μm and 2.40 μm). Moreover, specimens of *Paramecium caudatum* were found, which in a large majority were free from lead deposits but some contained lead particles (1.80 μm) near the macronucleus.

On day 23 of treatment, other effects worth mentioning were observed, the ciliates disappeared from the fractions containing 100, 500 and 1000 μg/l of lead acetate (3C, 4C and 5C). In fraction 2C, containing 5 μg/l of lead acetate, specimens of *Paramecium caudatum* survived, the majority of which showed a single lead deposit relatively large in size (3 μm) in the fore part of the body. The fraction 2D, with 300 μg/l of lead acetate, still had three species of ciliates on day 23, all presenting lead deposits (Fig. 9). One of these species was *Paramecium caudatum*, in which several lead deposits of different sizes were present, the largest (1.80 μm, numbering from 1 to 4) generally occurring near the macronucleus and the smaller (1.20 μm) being also found in a number of 1 to 4. Another species observed was *Histriculus similis*, the majority of which either completely lacked lead particles or contained a reduced number of small lead particles (less than 6, 0.30 and 0.40 μm

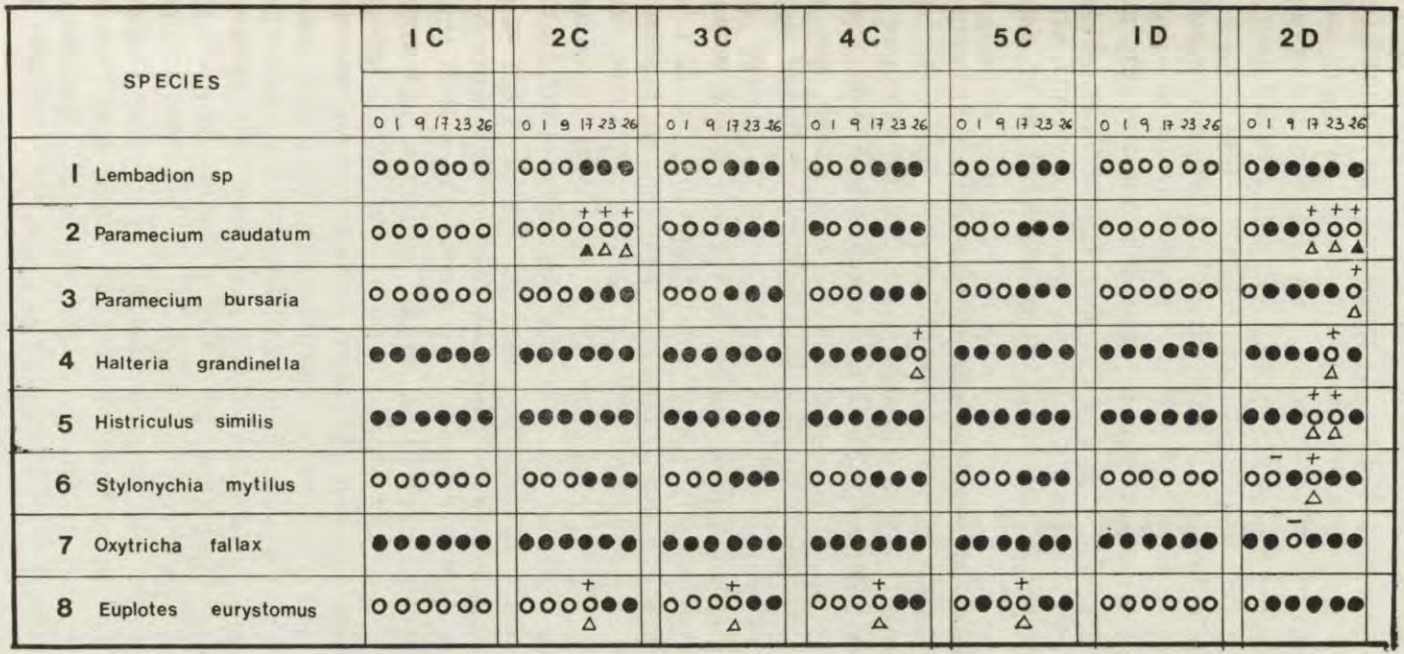

Fig. 9. Lead distribution in the different ciliate species, Ciudad Universitaria samples: + with lead, — without lead, filled triangle — bunching particles, triangle — non-bunching particles, species absent, dot — species present

in size). The third species was the oligotrich *Halteria grandinella* which had not been present in sample D till this date (Fig. 9). Lead particles in specimens of this species were generally situated under the ciliate's equator and above the ciliate's subequatorial belt. Particles (between 1 to 5) had a relatively small size (0.60 μm).

The following effects worth pointing out were observed on day 26 of treatment. In sample C, no ciliates were present in fractions containing 100 and 1000 μg/l of lead acetate (3C and 5C). Fraction 2C, with 5 μg/l of lead acetate, still presented specimens of *Paramecium caudatum* containing lead deposits generally found in the rear body half (Fig. 9). In the majority of the specimens, a large-sized bunch (1.20 μm) and 1 to 6 particles of small size (0.36 μm) were seen. In fraction 4C (500 μg/l of lead acetate), there was a considerable development of the oligotrich *Halteria grandinella*, the majority of these specimens presented lead particles situated in the rear body half. The particles, numbering 1 to 10, were relatively larger (2.25, 3 and 1.50 μm) than particles in the cells of the same species on day 23 of treatment in fraction 2D.

In fraction 2D, containing 500 μg/l of lead acetate, *Paramecium caudatum* and *Paramecium bursaria* were present on day 26, practically all specimens of these species had a single deposit of lead particles bunching differently in either species. The particles in *Paramecium caudatum* were distributed in bunches generally located in the fore left part of the body and near the anterior end of the macronucleus. Each bunch consisted of 6 to 8 lead particles of medium size (1.50 μm) and had an average diameter of 2.25 μm. Specimens of *Paramecium bursaria* presented many particles, 0.75 and 1.50 μm in size, distributed all over the ciliate cytoplasm, although preferably concentrated near the macronucleus and in the body rear half. In many specimens there were about 50 particles near the nuclear apparatus.

After day 26 of treatment, no ciliate protozoa occurred in the treated samples.

Discussion

Dynamics of the Ciliate Communities with and without Lead

(1) Santillana Reservoir (Fig. 8)

Nine of the encountered 12 ciliate species were preserved at least in the control fraction during the different observation times, and three species appeared only for a short time between sample collection and the day of lead acetate addition to the different fractions (*Amphileptus*

claparedei, *Litonotus lamella* and *Tachysoma pellionella*). Four species were present on day 0 in all fractions but disappeared from the community by day 6 (*Prorodon ovum*, *Frontonia leucas*, *Colpidium colpoda* and *Stylonychia mytilus*).

Of all species studied only *Vorticella microstoma* was present at all observation dates but only in control fractions (1A and 1B). In addition 4 species first appeared in control fractions by day 6 (*Paramecium bursaria*, *Keronopsis rubra*, *Stentor roeseli*, and *Histriculus similis*) and were preserved there to the last day of observation. In two species, the vegetative forms temporarily disappeared from some fractions but re-appeared later, on day 14: *Paramecium bursaria* in fraction 4A and *Stentor roeseli* in fraction 3A. Some species were found only on observation day 6, before addition of lead to the respective fractions: *Paramecium bursaria* in fractions 2A and 5A, *Stentor roeseli* in fractions 2A, 4A, 5A and 2B, *Keronopsis rubra* in fractions 2A, 3A, 4A, 5A and 2B, *Histriculus similis* in fractions 2A, 3A, 4A and 5A.

The control fractions showed the highest number of species (5) surviving to the last observation date.

On day 6, before lead acetate was added to the experimental fractions, only communities of five ciliate species could be observed there (*Paramecium bursaria*, *Vorticella microstoma*, *Histriculus similis*, *Keronopsis rubra*, and *Stentor roeseli*). After lead addition to fraction 3A, only two species survived: *Paramecium bursaria*, with loose lead particles, and *Stentor roeseli*, with bunching lead particles.

In fraction 4A, only one species (*Paramecium bursaria*) survived to lead addition, and presented clustered particles on day 14, while no species survived lead addition in fraction 5A to which the highest concentration was added. In fraction 2B, only two species survived lead acetate addition: one of them (*Histriculus similis*) till day 10 only, with no lead particles present, and the other, *Paramecium bursaria*, till day 17, presenting lead particles clustered only on day 14.

(2) Ciudad Universitaria (Fig. 9)

In the control fractions 1C and 1D, five species were mantained all along the period of observation: *Lembadion* sp., *Paramecium caudatum*, *Paramecium bursaria*, *Stylonychia mytilus*, and *Euplotes eurystomus*. Three other species, which did not occur in the samples on the day when lead was added to the media, appeared only later in experimental but not in control fractions (Fig. 9). *Halteria grandinella* was found on day 23 and 26 of treatment, and *Histriculus similis* on days 17 and 23 of treatment, both with non-bunching lead particles.

The ciliate communities in fractions 2C, 3C, 4C and 5C did not generally survive until day 17 after treatment, except for two species: *Euplotes eurystomus*, observed on day 17 with non-bunching particles, and *Paramecium caudatum*, surviving during the whole observation period but only in fraction 2C and presenting lead particles on days 17, 23 and 26. In other fractions of sample C (with larger lead concentration), *Paramecium caudatum* did not survive later than day 9. The number of species surviving the treatment or appearing after its beginning was higher in fraction 2D, where two species were still present on day 26: *Paramecium bursaria*, with lead particles bunching, and *Paramecium caudatum* with bunching particles. Two species in this same fraction were observed on day 23, containing lead: *Halteria grandinella* and *Histriculus similis*, and an only species showing lead reaches day 17 with an interruption in the appearance of vegetative forms on day 9 (*Stylonychia mytilus*).

Lead Accumulation

(1) Santillana Reservoir (Fig. 10)

The largest and smallest sizes of intracellular lead particles were both found on day 10, that is, 4 days after lead addition; both occurred in *Paramecium bursaria*, the largest in fraction 2B (6 μm) and the smallest in fraction 3A (0.30 μm). The largest variation in particle size was found on day 14, that is 8 days after lead addition: in *Paramecium bursaria*, they were 0.75, 1.50, 1.80, 2.25 and 3 μm in diameter in fractions 2B, 3A, 2B, 3A and 4A respectively, in *Stentor roeseli*, there were two sizes of particles: 0.60 and 1.20 μm, both in fraction 3A. On day 17, only one size of particles (3 μm) was found, corresponding to *Paramecium bursaria* in fraction 2B (Fig. 10).

Among these fractions, 2B shows a stable pH as from day 10, and the other two (3A and 4A) show a rising pH till day 17. In fraction 2B *Paramecium bursaria* showed lead particles from day 10 to day 17: large particles occurred on day 10, three types of particles on day 14, and large size particles on day 17. In this fraction, oxygen stabilizes as from day 14. *Paramecium bursaria*, surviving till day 17 and accumulating toxic particles in their cells, thus seems to be a safe lead indicator.

It is convenient to stress the effects of lead on two other species: *Histriculus similis*, appearing on day 10 and disappearing from that

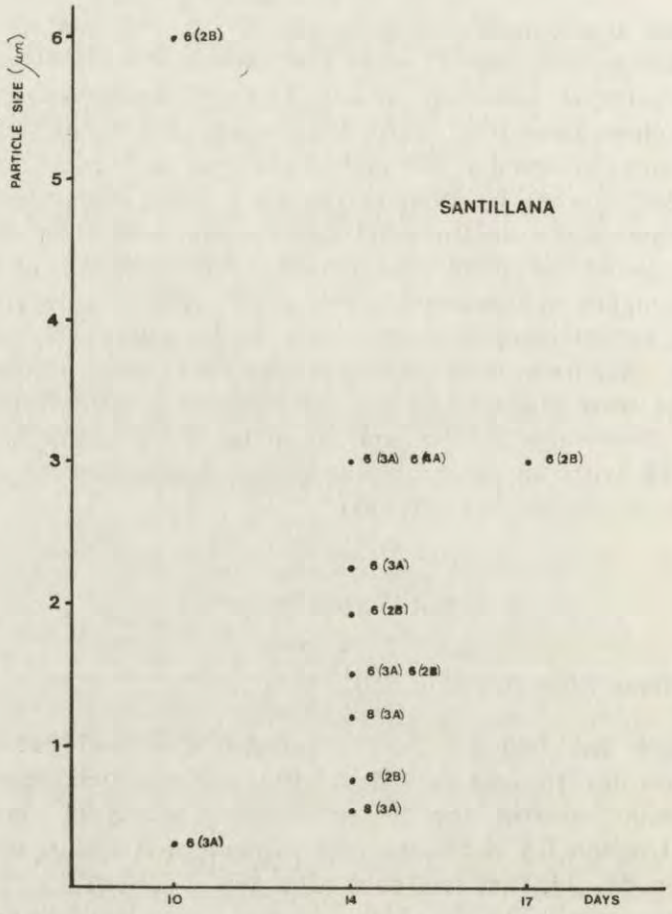

Fig. 10. Lead particles size in ciliates in the various fractions of Santillana samples, according to exposure time. The numeral before the fraction number designates the species concerned, numbered as in Fig. 8

day on, which fails to accumulate toxic particles, and *Vorticella microstoma* which, although being a safe indicator of the polysaprobic zone, does not survive lead acetate addition in any of the fractions.

(2) Ciudad Universitaria (Fig. 11)

The largest variation in lead particle size corresponds to day 17, with particles varying from 0.30 μm (in fraction 5C, in *Euplotes eurystomus*) to 6 μm in fractions 4C and 5C in the same species. This range of variation corresponded to the largest diversity of species observed in the communities (*Euplotes eurystomus*, *Paramecium caudatum*, *Stylonychia mytilus*, and *Histriculus similis*).

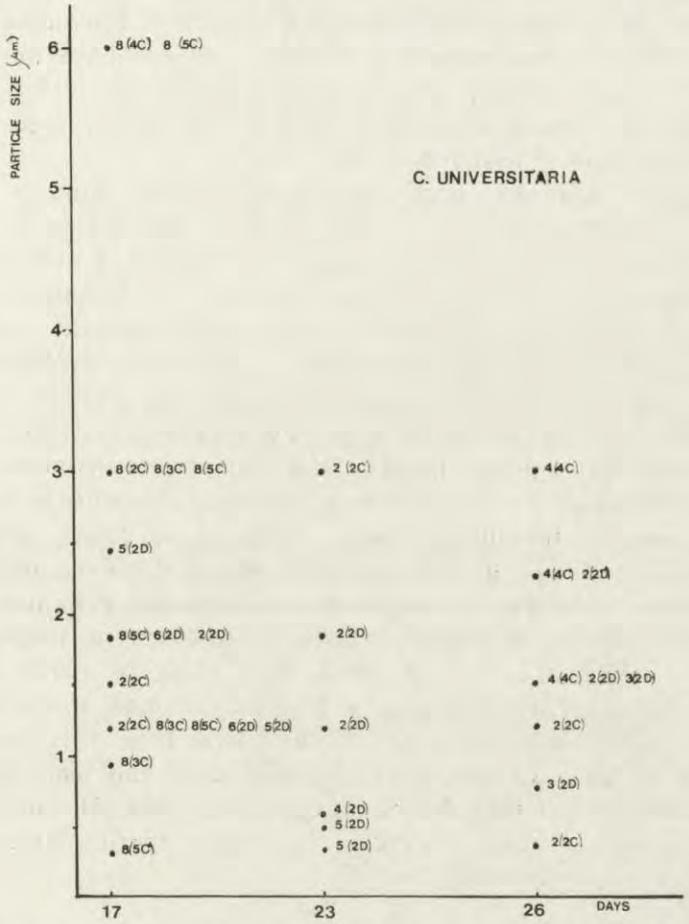

Fig. 11. Lead particles size in ciliates in the various fractions of Ciudad Universitaria samples, according to exposure time. The numeral before the fraction number designates the species concerned, numbered as in Fig. 9

At other observation dates when lead particles were found, their size did not surpass 3 μm (Fig. 11).

On day 23, the size of particles varied between 0.30 and 3 μm in *Histriculus similis*, *Halteria grandinella*, and *Paramecium caudatum*. On day 26, the particle size was within the same range in *Paramecium caudatum*, *Paramecium bursaria* and *Halteria grandinella*.

Lembadion sp. does not survive after day 9 in any of the fractions containing lead, and in 2D after day 1.

Paramecium caudatum normally appears in fractions showing variably sized lead in its interior, and on day 26 in fraction 2D it appears bunching. Under these conditions, *Paramecium caudatum* is a good indicator of lead pollution. In this fraction there were, moreo-

ver, *Paramecium bursaria*. In the samples of Ciudad Universitaria there was a high organic contamination providing as adequate medium for *Paramecium bursaria*, which is a meso-polysaprobic indicator. On lead being added, this ciliate disappears from day 9, appearing once again on day 26 and showing lead in its cell.

Another good indicator with respect to exposure time is *Euplotes eurystomus*, a species which survives in lead containing fractions of sample C till day 17. On day 9 it appears without lead and on day 17 shows bunching lead particles. After this date, it disappears. In fraction 2D it probably fails to survive owing to competition with other species, especially those appearing after a relatively prolongated exposure time, such as *Halteria grandinella*, which in fraction 4C substitutes all other species (*Lembadion* sp., *Paramecium bursaria*, *Paramecium caudatum*, *Stylonychia mytilus* and *Euplotes eurystomus*).

Lead accumulation by the ciliates in the samples seems to be related with the amount of measured oxygen. This can be clearly appreciated in fraction 2D (500 μg/l of lead acetate), where dissolved oxygen stabilizes from day 1 to day 9, and increases from day 9 to day 17. The appearance of ciliates on day 17 causes a reduction of the remaining organic mass which they use as food, thus bringing about a stabilization in oxygen production and a transfer of lead to ciliate cells. Some of these disappear after day 23, and it is from this day oxygen production rises with an increase in organic mass and lead absorption; and it is exactly in this period that pH increases. This pH value dropped during the period of lead absorption by ciliate species (from day 17 to 23).

Conclusions

(1) The presence of lead in water at 100 to 1000 μg/l concentrations determines a series of effects: (a) on organic matter in general, (b) on ciliate protozoan communities, (c) on certain physico-chemical factors.

(2) The affect on ciliate protozoan communities is appreciated in: (a) lead particle accumulation by the organism, either bunching or not, and situated in distinctive zones of the cell, (b) disappearance of species under specific time of exposure and specific toxic concentration, (c) substitution of species within the community.

(3) Effects on physico-chemical conditions are to be seen in: (a) dissolved oxygen concentration variations, (b) pH variations related with (a).

(4) Ciliate species can be assayed as indicators in different ways:

(a) by their disappearance according to exposure time and/or toxic concentration, (b) by quantity, size, clustering and localization of toxic particles in their cells, (c) by their interaction with other ciliate species. Several examples of this type are shown in this study.

(5) The use of ciliates as bioindicators of lead contamination is possible if appropiate species are employed, which would in practice not only allow the knowledge of the effects of toxics on communities but, moreover, would avoid the use of expensive valorization methods. On the other hand, the latter would give no clear idea on ecological conditions of water zones.

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ACTA PROTOZOOLOGICA
Vol. 24, No. 2, pp. 111-116 (1985)

Studies on the Role of External Na^+ in the K^+-induced Ciliary Reversal in *Fabrea salina*

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Received on 15 October 1984

Synopsis. Removal of Na^+ from the external medium prolonged K^+-induced ciliary reversal in *Fabrea salina* and had the direct effect on the repolarization phase of the membrane potential. It is suggested that the absence of Na^+ in the medium could decrease or abolish Ca^{2+}-Na^+ exchange system which is responsible for an extrusion of Ca^{2+} from the intraciliary space and makes a contribution to the resting potential.

Fabrea salina is a marine ciliate, grown in highly concentrated salt solution containing 1.1 M NaCl. Sodium chloride can be replaced in the medium by choline chloride in the osmotically equivalent amount without any visible behavioural changes in the ciliates (Kubalski 1983 a). In the present study the duration of K^+-induced ciliary reversal (CR) in the standard medium (containing Na^+) and in the Na^+–free medium was compared. Parallel electrophysiological studies on *Fabrea salina* were performed. The membrane potential was recorded while the cells were transferred to enriched in 0.05 M KCl standard and Na^+-free solutions.

As it was shown on *Paramecium calkinsi* (Deitmer and Machemer 1982) and *Fabrea salina* (Kubalski 1983 a) the excitability of marine ciliates is similar as in fresh water ciliates — Ca-dependent, although the large concentration of Na^+ in external medium plays an important role in their membranes properties. The aim of present study was to add some new data to the results achieved earlier by the mentioned authors on the effects of Na^+ on the excitability of marine ciliates.

Material and Methods

Fabrea salina strain (obtained from the Laboratory of Zoology, Ecole Normale Superieure, Paris) was grown in the medium: 1.1 M NaCl + 0.13 M $MgCl_2$ + 0.07 M Na_2SO_4 + 0.025 M $CaCl_2$ + 0.022 M KCl + 0.005 M $NaHCO_3$ + + 0.005 M Tris/HCl (pH 7.2) with addition of *Aerobacter aerogenes* as standard food supply. Cells used for experimentation were kept in unbacterized medium for 1-2 days. The standard control solution was:
1.1 M NaCl + 0.13 M $MgCl_2$ + 0.025 M $CaCl_2$ + 0.022 M KCl + 0.005 M Tris/HCl (pH 7.2).

In all experimental solutions the ionic strength was kept constant. When a concentration of any component was increased the NaCl concentration was decreased by the osmotically equivalent amount. The Na-free solution contained choline chloride instead of NaCl.

The direct observations of behaviour were done under low magnification of optical microscope.

Electrophysiological recordings of membrane potential were carried out in the way and on the set-up described elsewhere (K u b a l s k i 1983 a). Membrane potential was measured first in control standard medium which was then replaced by perfusion with various test solutions. The last measurement of membrane potential was performed also in the standard control medium. The experimental chamber was perfused 4-5 times during an exchange of each test solution, by simultaneous inflow and removal of bath solution. The differences of the reference potential level in various test solution were 1-2 mV and they are not marked on Fig. 1 and 2.

Results

Introduction of K^+ ions to the medium always caused depolarization of the membrane and evoked ciliary reversal (CR). As it was published (D r y l et al. 1982) *Fabrea salina* responded with CR when the concentration of KCl was higher than 0.13 M (in the presence of 0.1 M $CaCl_2$) in the standard medium and showed ever-lasting CR when concentration of KCl was higher than 0.25 M. In the standard medium the threshold concentration of KCl for evoking of CR was about twice as high as in the Na^+-free medium (D r y l and K u b a l s k i, unpublished). The duration of CR in the both experimental solutions (standard and Na^+-free) evoked by the same KCl concentration was also different (see Table 1). It was noticed that the threshold concentration of KCl for induction of ever-lasting CR in Na^+-free solution was below 0.2 M KCl.

Electrophysiological recordings of the membrane potential were done when the concentration of KCl in the medium was 0.05 M (Fig. 1 and 2). As indicated in Table 1, in the presence of 0.05 M KCl, the duration

Table 1

The duration of ciliary reversal response induced by K^+ in standard medium and in Na^+-free medium

Concentration of KCl (M)	Duration of CR in standard medium (in min)	Duration of CR in Na^+-free medium (in min)
0.025	< 1	5
0.050	< 1	9
0.075	1	28
0.100	6	71
0.200	63	ever-lasting CR

The time of duration of CR was measured in direct observations and the data mentioned in the Table were established when more than 50% of tested specimen showed forward movement. The number of observations was not less than 5.

of CR in standard medium was shorter than 1 min and in Na^+-free solution CR lasted 9 min. Repolarization of the membrane potential in the medium containing 0.05 M KCl is shown in Fig. 1. Transfer of the cell into standard medium (marked with arrow "control") was connected with a short hyperpolarization and increase of a background noise. A large hyperpolarization of the membrane after removal of Na^+ from the medium is shown in Fig. 2. Addition of 0.05 M KCl depola-

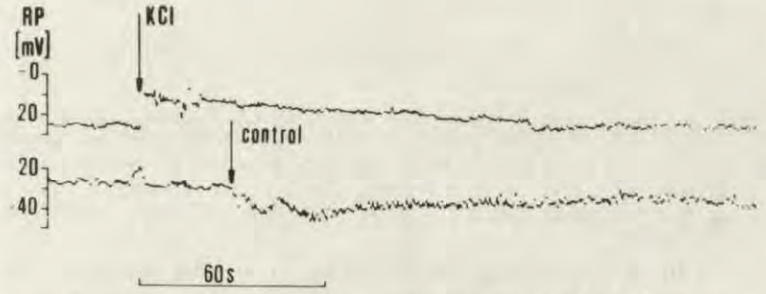

Fig. 1. The changes of the membrane potential of *Fabrea* in standard medium after addition of 0.05 M KCl (arrow "KCl"). The cell was next transferred to the standard medium (arrow "control"). The lower trace is a continuation of the upper one. They both show 7 min recording

rized the membrane in a degree more or less the same as in the case of standard solution (up to $\cong -10$ mV). The phase of long repolarization of the membrane potential came towards more positive values. Removal of K^+ (arrow "choline chloride") is again followed by hyperpolarization, very long lasting "action potential", next hyperpolarization and finally the membrane potential reaches its resting value between

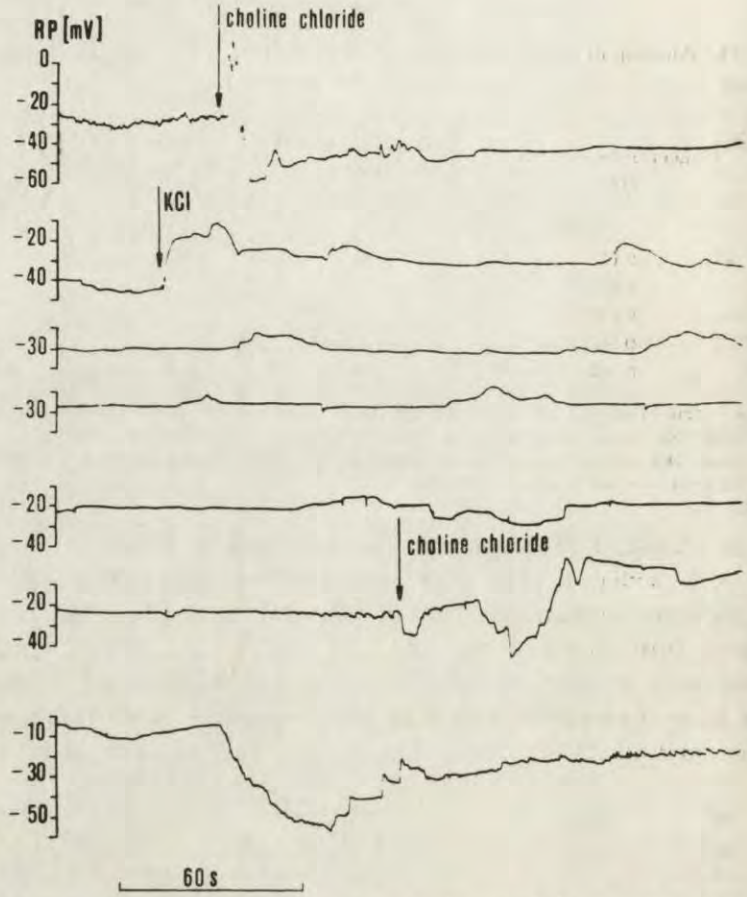

Fig. 2. The changes of the membrane potential of *Fabrea salina* when it was transferred from the standard medium to the Na+-free solution (arrow "choline chloride" on the top), then 0.05 M KCl was added to the Na+-free medium (arrow "KCl") and next KCl was removed from the Na+-free solution (arrow "Choline chloride" on the bottom). All the traces represent continual recording (25 min)

−20 and −30 wV (although still in the medium without Na^+). It is not shown in Fig. 2 but the retransferring of the cell back to the standard medium was followed by a short period of depolarization and return to its resting value.

Discussion

The restoration of normal ciliary beating occurs when $[Ca^{2+}]_{in}$ is reduced (Eckert 1972, Naitoh and Kaneko 1973). Among various possible mechanisms there is a possibility of Ca^{2+} extrusion

from the intraciliary space into outside. It was suggested (Browning and Nelson 1976) that Ca^{2+}-Na^{+} exchange pump detected and described in squid axons (Baker et al. 1969, Blaustein 1974), barnacle muscle (DiPolo 1973) and other membranes (Langer 1982, Connolly and Kerkut 1983 — reviews) may also play a marked role in reduction of intraciliary Ca^{2+} during excitation in *Paramecium*. The results presented by Hansma (1979) and Saimi and Kung (1980) demonstrated prolonged inward Na^{+} current through the ciliary membrane which was Ca^{2+}-dependent and associated with backward swimming in paranoiac mutants of *Paramecium*.

It was found in the marine ciliates that replacing sodium ions in the medium with choline chloride made the membrane potential more negative. In the case of *Paramecium calkinsi* the membrane potential value in standard artificial seawater (0.436 M NaCl) was −10 mV (±1 S.D.) and in Na^{+}-free seawater its value was between −25 mV −32 mV (Deitmer and Machemer 1982). The membrane potential in *Fabrea salina* measured in standard control medium (1.1 M NaCl) was −32 mV (±4 S.D.) and in Na^{+}-free medium its value fell down up to −80 mV (Kubalski 1983 a). In the both mentioned cell populations the input resistance increased too. Those data and the prolongation of induced CR in the absence of external Na^{+} indicate that Ca^{2+}-Na^{+} exchange system may operate in marine ciliates and seems to be involved in restoration of normal ciliary beating during excitation.

Further prolongation of the CR response induced by K^{+} can be realized by addition to the medium K^{+}-conductance antagonist — tetraethylammonium (TEA) what is in agreement with results achieved on fresh water ciliates (Satow and Kung 1976, Doughty and Dryl 1981). In the standard medium containing 0.01 M TEA+0.1 M KCl the duration of CR was 11 min and in the Na^{+}-free solution (containing the same amounts of TEA and KCl) CR did not disappear after 2 h (Kubalski, unpublished).

The changes of the membrane potential in the conditions of K^{+}-induced, long lasting ciliary reversal in *Fabrea salina* were demonstrated elsewhere (Kubalski 1983 b). Presented results may indicate that the removal of Na^{+} from the experimental medium caused hyperpolarization which is partly reduced spontaneously by the membrane itself. Addition of low concentration of KCl to the Na^{+}-free solution was accompanied by rapid depolarization of the membrane potential. Immediately after introduction of KCl into the medium, the membrane potential value was more or less the same in the both experimental solutions (standard and Na^{+}-free). The process of repolarization of the

membrane potential (i.e., recovery of its resting value) in the presence of K^+ in the medium was much slower in Na^+-free solution than in the standard one.

Summarizing — removal of Na^+ in the external medium prolongs K^+-induced CR in *Fabrea* and also has an effect on repolarization of the membrane potential. It would be too simple to join these two events together (there are more components influencing each of these processes — for example presented effect of TEA on CR duration) but it can be assumed that in Na^+-free solution extrusion of Ca^{2+} into outside by Ca^{2+}-Na^+ exchange pump is decreased or abolished, and Ca^{2+}-Na^+ exchange system may be considered as an electrogenic one, contributing to the resting potential.

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ACTA PROTOZOOLOGICA
Vol. 24, No. 2, pp. 117-124 (1985)

Changes in the Behaviour of *Paramecium caudatum* Caused by Incubation in Various Cholesterol Concentrations

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Received on 9 October 1984

Synopsis. The influence of cholesterol present in the liposomes on the behaviour of *Paramecium caudatum* cells was tested. The role of cholesterol, as factor evoking changes in biological membranes, mentioned by numerous investigators was confirmed in the present experiments. Cells incubated in various lipid concentrations exhibited disorders in the physiological functioning of the cell membrane manifested in a prolonged duration of ciliary reversal. The observed changes were probably the consequence of an increased microviscosity of the membrane, leading to a limitation of active and passive transport through the biological membrane.

Several functional properties of the cell membrane are dependent on its structure, organization and fluidity of the lipid double layer. Such functions of the cell as growth (Machtiger and Fox 1973), active transport and respiration (Overath et al. 1970) are largely dependent on the physical state of membrane lipids. Probably the mechanism lying at the base of these vital processes is connected with the interaction of proteins and lipids, and this is affected by the composition of phospholipids and concentration of the particular lipid components of the membrane. Neither can the role of the cholesterol/ /phospholipid ratio be disregarded since it seems to be an important determinant in the regulation of the biological properties of the membrane (Demel et al. 1976).

In the biological membranes built of a mixture of various phospholipids with different degrees of saturation of fatty acids, the effect of the presence of cholesterol on the orientation group arrangement, rotational and linear diffusion of lipid molecules is functionally very important. A consequence of changes in the concentration of this sterol

in the membrane is a change in the fluidity and viscosity of the double lipid layer and disturbance in the rotational and linear movement of lipid molecules within these layers (Kruijff 1974).

These changes bring about disorders in passive and active transport (Madden and Quin 1979, Madden et al. 1981, Drabikowski et al. 1972, Wiley and Cooper 1975, Kroes and Ostwald 1971, Cooper et al. 1975), and this in turn lies on the base of processes of cell excitability.

By modifying the cell membrane, increasing or reducing its permeability, a distinct reaction can be evoked, manifested in the case of *Paramecium* by a protraction of duration of the ciliary reversal (Szydłowska-Fabczak 1981, 1983 a, b).

Although cholesterol is not characteristic of ciliates (Conner et al. 1971), previous studies (Szydłowska-Fabczak 1981, 1983 a, b) demonstrated that the presence of cholesterol in the cell membrane of these protozoans causes an effect comparable to the action of this sterol in cells of higher animals, where it is vitally indispensable component of the membrane.

The use of liposomes enables incorporation of cholesterol into the membrane of the wild stock of *Paramecium caudatum*. It allows at the large degree to eliminate the changes in the metabolism of the cells due to culture medium containing cholesterol (Conner et al. 1982).

On the basis of the processes of excitability in ciliates and ion exchange throughout the cell membrane connected with these processes (Machemer and Eckert 1975, Naitoh and Kaneko 1972, Eckert 1972, Conolly and Kerkut 1983), it was decided to investigate the changes in the physiology of the cell behaviour of *Paramecium caudatum* under the influence of various concentrations of cholesterol contained in liposomes.

Material and Methods

Liposomes were obtained by the method of Bangham et al. (1965). Cholesterol and phosphatidylcholine (lecithin) were dissolved in chloroform in a round-bottom flask, in molar ratio CH : CP 1 : 1 or 0 : 1. Chloroform was evaporated under reduced nitrogen pressure at room temperature. To the lipids forming a thin layer at the bottom of the flask a buffer solution was added (1 mM Tris HCl, 1 mM $CaCl_2$, pH 7.3). The suspension formed was shaken at 37°C for about 30 min, under nitrogen atomosphere, until the lipids were completely removed from the flask walls. Then the suspension was subjected to the action of ultrasounds, until the clear solution was obtained. Sonication was run under the nitrogen atmosphere for 2-5 min with 30 s intervals in the water bath at 0°C on an ultrasound disintegrator (MSE, vibration amplitude 3 μm). The

obtained liposomes preparation was incubated for 1 h at room temperature and then centrifuged at 60 000 × g for 20 min. The middle liposome fraction was used for the experiments.

Ciliates used in this study were *Paramecium caudatum* (wild strain) isolated from water near Warsaw in 1955 and kept in mass cultures on the standard lettuce medium inoculated with *Aerobacter aerogenes* (Sonneborn 1950).

On the day preceding the experiment, the ciliates were washed and concentrated by threefold centrifugation at 800 × g in buffer solution at room temperature and left to stand for 12 h. Then ciliates were incubated with the liposomes suspension in such appropriate proportion as to obtain a final cholesterol concentration in the particular samples of 0.005, 0.01, 0.05, 0.1 mM respectively. After 2 or 4 h of incubation the paramecia were washed by centrifugation in buffer solution in order to remove paramecia from medium containing liposomes. A drop of liposome treated paramecia (about 5 μl) was introduced into 2 ml of solution evoking ciliary reversal consisting of KCl in the concentration ranging from 20 to 60 mM, dissolved in buffer solution. The choline chloride was added to this solution in a proper amount to maintenance of a constant ionic strength at the 66 mM level. Duration of ciliary reversal induced by K^+ ions was measured by means of stop watch.

Results

The incubation of the cells of *Paramecium caudatum* in medium containing cholesterol-enriched liposomes indicates a distinct influence of cholesterol on duration of ciliary reversal (CR), caused by extracellular increase of K^+ concentration, which is considered here as an index of cell membrane excitability (Table 1 and Fig. 1). The K^+-induced duration of CR was proportional to the liposomal cholesterol concentration in the concentration range from 5×10^{-5}M to 10^{-3}M at room temperature after 2 h of incubation. Computer analysis for curve fitting was performed with a programmable Hewlett-Packard HP-33E calcu-

Table 1

Duration of ciliary reversal (CR) of *Paramecium caudatum* induced by increases of K^+-ion concentration after incubation in suspension of liposomal cholesterol (CH:CP = 1:1). Time was measured in seconds

Cholesterol concentration	20 mM KCl	30 mM KCl	40 mM KCl	50 mM KCl	60 mM KCl
Control	15±1	21±2	33±3	39±4	47±2
0.005 mM	17±3	25±1	37±1	44±2	51±1
0.01 mM	19±1	28±1	41±2	50±4	59±5
0.05 mM	21±2	31±2	45±4	57±2	66±4
0.1 mM	25±3	37±1	51±3	64±5	73±3

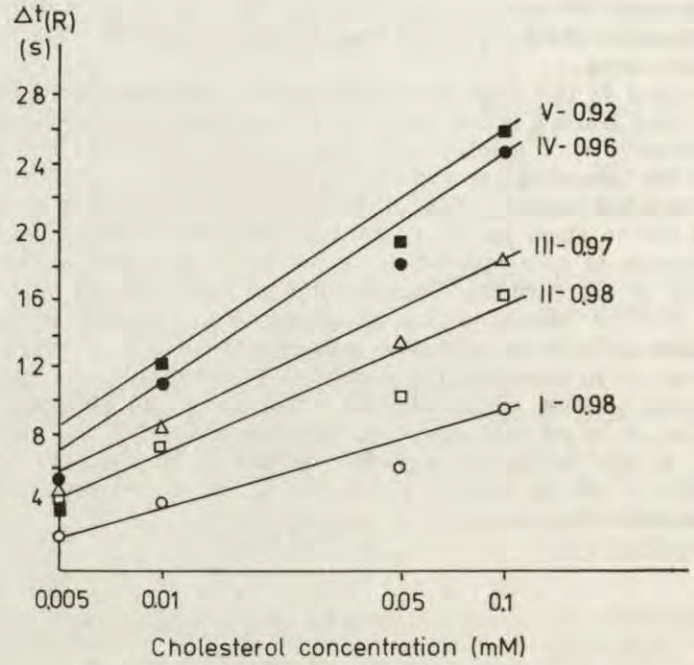

Fig. 1. Effect of treatment with cholesterol-enriched liposomes (CH : CP 1 : 1) on the duration of CR (Δt) in *Paramecium caudatum* caused by different K^+-concentrations (I — 20 mM, II — 30 mM, III — 40 mM, IV — 50 mM, V — 60 mM). The data are taken from Table 1

lator. The correlation coefficients (r^2) for linear fitting were above 0.93. The CR duration was also linearly increased with time over four hours of time course experiment (Fig. 2). The effect of cholesterol on the lasting of cell CR for protozoan cells tested at different concentration of liposomal cholesterol is shown in the Fig. 1. The values of CR duration increase at a maximum of about 30 to 50% of their control values (i.e., cells not treated with cholesterol) induced by the changes in the extracellular K^+-ion concentration from control level to chosen one (2 to 6×10^{-2}M).

To exclude possible direct influence of lecithin on the K^+-induced cell excitability (i.e., duration of CR), the separate control experiments were performed in which the protozoans were incubated with liposomes containing pure lecithin. As seen in Table 2, there are no significant changes in CR duration with respect to non-treated control cells. In this case rather slight shortening than prolongation of CR was observed. The CR duration is prolongated only when cholesterol was present in the phospholipid vesicles.

In order to elucidate whether CR modulations caused by choleste-

Table 2

Duration of ciliary reversal of *Paramecium caudatum* induced by increases of K^+-ion concentration after incubation in suspension of liposomes (CH:CP = 0:1). Time was measured in seconds. Concentration of lecithine was 0.1 mM

Incubation time	20 mM KCl	30 mM KCl	40 mM KCl	50 mM KCl	60 mM KCl
Control	15±1	21±2	33±3	39±4	47±2
2 h	11±2	18±2	27±3	32±1	41±3

rol are reversible, protozoans previously incubated with cholesterol over 2 h and demonstrating prolongated CR duration were incubated with egg-lecithin liposomes to remove the cholesterol added. A statistically significant decrease of the CR duration was observed; this clearly indicates that the excitability modulation in paramecium cells caused by cholesterol is reversible (data not shown).

Discussion

Numerous experimental data confirm the supposition that the appearance of the ciliary reversal (CR) in *Paramecium* cells under the influence of the external stimuli is the result of depolarization of cell membrane, influx of calcium ions into the cell through calcium channels located within the ciliary membrane (Dunlap 1977). The duration of CR and cell backward swimming is dependent on the time of opening of Ca^{2+} channels (Dryl and Hildebrand 1979) and on the rate of removal of the cytoplasmic Ca^{2+} excess by an active way (Conolly and Kerkut 1983).

The results of the excitability measurement shown in Table 1 and Fig. 1 indicate that the incubation of *Paramecium* cells with liposomal cholesterol modifies greatly the behaviour of cells as the result of incorporation of the cholesterol into membrane. In this work no attempt to measure the amount of cholesterol effectively incorporated in the cell membrane were made. However, as the cholesterol to phospholipid ratio (CH : CP) equals zero in the cell membrane of *Paramecium* (cholesterol is not a parmanent membrane component) (Conner et al. 1971), liposomal preparations having a CH : CP ratio of 0.5 or more would produce a cholesterol enrichment (Shapiro and Barchi 1981). The presence of cholesterol in the cell membrane prolongs the time of backward swimming (i.e., CR duration) under the K^+-ion stimulus, without any detectable changes in the other parameters of the

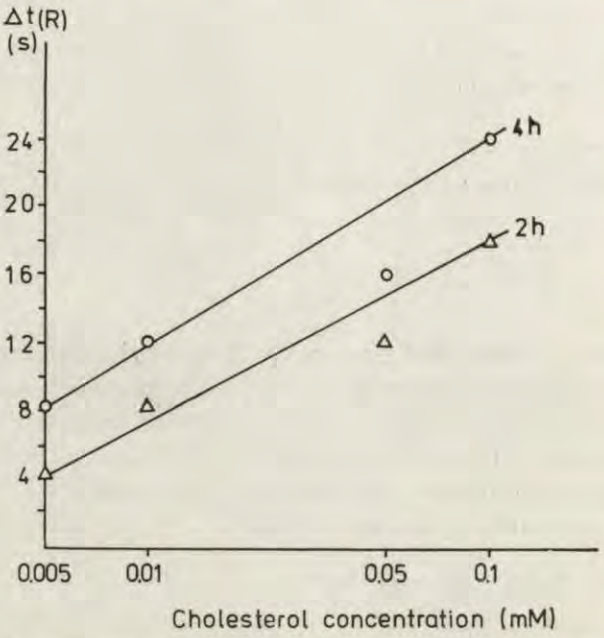

Fig. 2. Effect of incubation time (i.e., 2 and 4 h) on the excitability (CR) of *Paramecium caudatum*. The duration of CR at the same chemical stimulus (K+-concentration — 40 mM)

cell behaviour (i.e., forward swimming). This phenomenon is dependent not only on the presence of cholesterol in cell membrane but on the liposomal cholesterol concentration and incubation time as well. As seen in Fig. 2 the prolongation of CR duration (Δt), increases proportionally to the concentration of liposomal cholesterol over concentration range tested. Prolonged treatment of *Paramecium* cells with cholesterol-enriched liposomes caused increase in value of CR duration (Fig. 1). The control magnitude of CR duration of the cholesterol-enriched cell of *Paramecium* could be restored on subsequent incubation of the cells with lecithin liposomes for relatively short time (less than 1 h) (unpublished data). This treatment, which presumably deplets the membrane cholesterol in a similar fashion to that observed in other cells (Cooper et al. 1975, Shinitzky and Inbar 1976).

Zyzek et al (1983) while investigating the passive and active properties of pituitary cells (GH3/B6) obtained changes in the membrane potential, shape and amplitude of the recorded action potentials after incubation of the cells in liposomal cholesterol suspension with CH : CP up to value of one. This effect of cholesterol on excitability of pituitary cells similarly as in case of *Paramecium* cells on the behavioural data, presented here, were reversible. It has been shown, that alterating mem-

brane cholesterol content affects the microviscosity of the membrane (Shapiro and Barchi 1981) and thereby modifies the mobility of membrane enzymes and transport channels.

Results reported here suggest that the membrane ionic channels associated with passive and active electrical properties of protozoan cells, *Paramecium caudatum*, are also affected by changes of membrane cholesterol content. In principle, decrease in membrane fluidity can also be achieved by reducing the temperature. Actually it has been shown, that by lowering temperature, the CR duration is prolonged, but to higher extent than with cholesterol enrichment (Hildebrand 1978). Possible alteration of the Ca^{2+}-ATPase activity in the cholesterol enriched membrane in protozoan could be the factor which mediate the observed changes in the cell behavioural activity (Browning and Nelson 1976, Doughty 1978). Cholesterol adsorption on the membrane vesicles of the endoplasmic reticulum from rabbit leg and back skeletal muscle cells depress both the Ca^{2+}-dependent ATPase activity and Ca^{2+} uptake simultaneously (Drabikowski et al. 1972). Thus, the observations presented here and those which have been previously published (Szydłowska-Fabczak 1981, 1983 a, b) suggest that an experimental enrichment of membrane cholesterol in *Paramecium* cells affects the cell behaviour activity by prolonging of the renormalization phase of the ciliary beating, due to changes in Ca^{2+}-ATPase activity and alteration of internal Ca^{2+} level simultaneously.

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ACTA PROTOZOOLOGICA
Vol. 24, No. 2, pp. 125-133 (1985)

Oscillatory Nature of Medium Acidification by Plasmodium *Physarum polycephalum*

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Received on 9 October 1984

Synopsis. Studies conducted with the use of the technique described below (H^+-selective glass microelectrode, fluorinert liquid) reveal an oscillatory process of medium acidification by the veins of plasmodium *Physarum polycephalum*. The medium pH is strongly dependent on ambient temperature. In a lower temperatures the acidification process itself is less intensive and the oscillations of pH value are slowed down. It was presumed that electric fluctuations appearing on the plasmatic membrane of the plasmodium veins are caused by observed flows of H^+-ions through the membrane. The periodic local accumulation of protons at the membrane surface, resulting from the functioning of metabolic mechanisms, as proved by its dependence on temperature, can bring about essential changes of electrochemical properties of the membrane, thereby affecting the membrane potential.

The oscillating electrical activity in the plasmodium myxomycetes was studied extensively by many authors. Watanabe and his coworkers (1937) found that if two glass micropipettes 20-30 μm in diameter were inserted at any two sides on a plasmodial vein of *Didinium nigripes*, the electrical potential difference fluctuated rhythmically along its length. Introduction of a simple "double chamber" method by Kamiya and Abe (1950) to measure cell surface potentials in the acellular slime mold *Physarum polycephalum* initiated numerous electrophysiological investigations of this organism. All the observations, including those using intracellular microelectrode technique for the measurement of electrical potential difference between the plasmodium protoplasm and the environment (Rhea 1966, Tauc 1954, Miller et al. 1968, Meyer and Stockem 1979) proved the existence of

rhythmical electric changes in *Physarum*. Using the fluorinert liquid method (Chen 1977, Meyer and Stockem 1979) showed clearly that electrical oscillations are present in the plasmodium and may be due to "rhythmic ion fluctuations between the slime mold protoplasm and its environment...". This suggestion seems to be valid in the light of a recent discovery that free Ca^{2+} and/or H^+-ions are involved in protoplasmic fluctuations.

The intention of this work is to investigate whether H^+-ions are responsible for generating rhythmical electrical changes in the vein of *Physarum polycephalum*. Some preliminary results have been presented elsewhere (Fabczak and Fabczak 1984).

Material and Methods

Plasmodia. Stock cultures of *Physarum polycephalum* were maintained on wet oatmeal on agar or filter papers in semidarkness at room temperature (Camp 1936). The cultures were kept a week or longer by daily refreshing the oatmeal.

Measurement procedure. For experiments, plasmodial veins about 0.5 mm in diameter and 2 cm in length with no branch were isolated and dried in air for 1 or 2 min. Subsequently the veins were placed in a teflon chamber with fluorinert liquid (FC-43 or FC-77, 3M Company) and mounted on a stereoscopic microscope stage. One or two small droplets of distilled water were deposited half-way along vein fragment. A specific selective glass microelectrode was inserted in the droplet to measure H^+-ion activity with the aid of a pneumatic micromanipulator. Temperature in the chamber was controlled by feedback Peltier's device within 0 to 20°C. Additionally, bath temperature was monitored by a solid state thermistor (Bauart type Gx 613) calibrated against a Hg-thermometer. The shuttle streaming of protoplasm in the fragmented vein was observed simultaneously by light stereo-microscopy. The change of protoplasm direction streaming was marked on the same chart as the external H^+-ion activity.

Microelectrodes. Conventional and H^+-selective glass microelectrodes were pulled from aluminosilicate capillaries (W-P Instruments) with a vertical puller. The capillaries were subsequently filled with a solution of 0.5 M NaCl buffered to pH of 7 with 0.1 M citrate buffer and bevelled to an angle of 45° until an electrical resistance of 10^7 ohms was reached (Ogden and Citron 1978). The micropipettes, after siliconizing, were filled with H^+-exchanger fluid (IE 100, W-P Instruments). Construction details of ion specific electrodes can be found in Thomas's monograph (1978). The micropipettes containing non-buffered solution of 0.5 M NaCl were used as reference microelectrodes. Both electrodes were fitted to a differential amplifier via Ag/AgCl half-cells. The time constants for the response of H+-selective microelectrodes to solution changes was 5 to 15 s. The H^+-selective microelectrodes were calibrated by running them through buffer solutions of various pH values, ranging from 6.0 to 8.0, adjusted either with 1 M HCl or 1 M NaOH (Fig. 1 A). The electrical resistance of H^+-selective microelectrodes at the same pH (7.0) was usually about 10^{12} ohms.

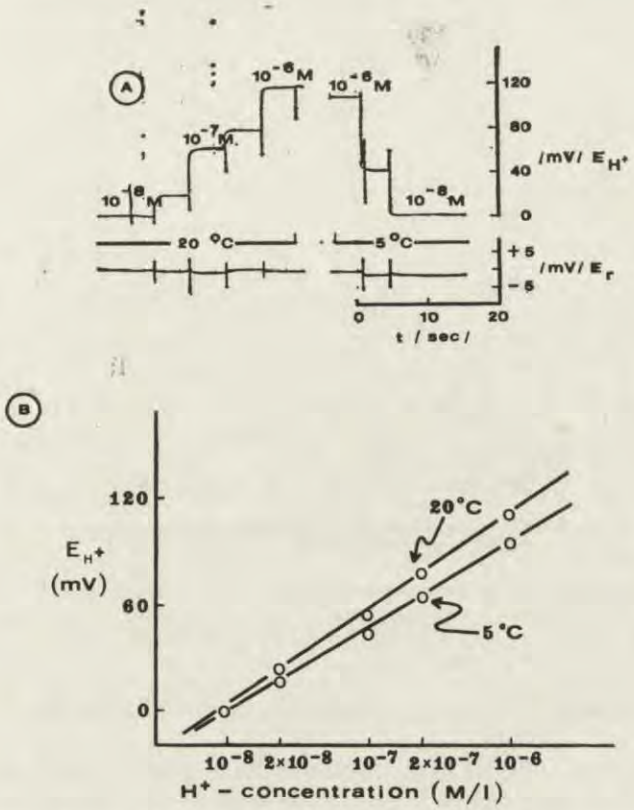

Fig. 1. Calibration traces (A) and graph (B) for H^+-selective microelectrode. The voltage responses of both the reference (E_r) and H^+-selective microelectrodes (E_{H^+}) were monitored simultaneously. The voltage recordings of E_{H^+} from (A) were plotted against the log (H^+)-concentrations (B)

Electric circuit. Figure 2 shows the arrangement for the measurement of H^+-ion activity in the droplet. A Burr-Brown Fet operational amplifier (A_2, type 3528, input impedance 10^{15} ohms) was used as differential preamplifier working at unity gain. A driven shield system was used, having aluminium foil wrapped around microelectrodes and connected to the output of the preamplifier. A continuous monitoring of H^+-ion activity (i.e., pH) was performed with a pen recorder or slow-sweep oscilloscope display.

Results

The isolated *Physarum* veins placed in fluorinert fluid showed after 15-20 min adaptation period, stable and regular oscillations of the level of H^+-ions in a drop of medium situated on the vein surface. Both insulating fluids used in the experiments (i.e., FC-43 or FC-77)

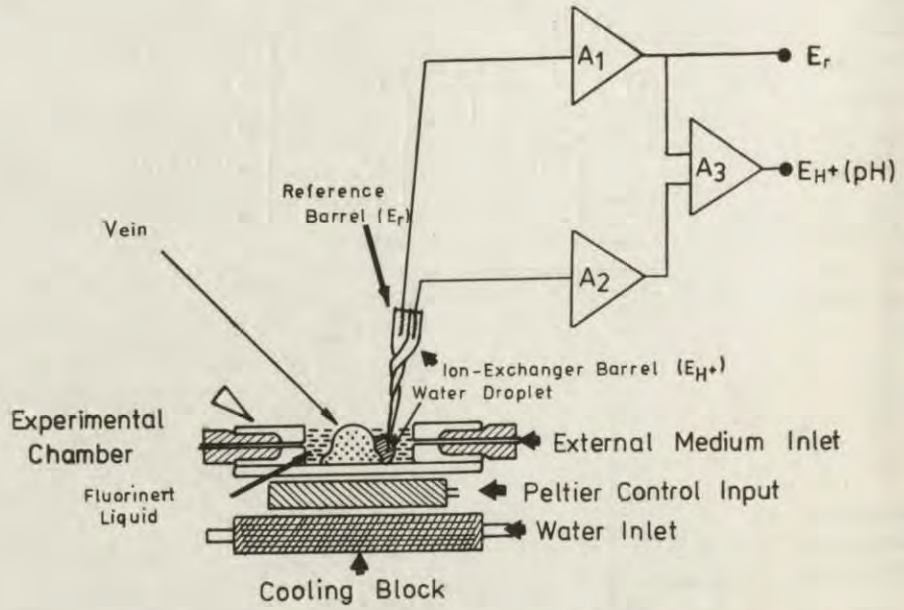

Fig. 2. Block diagram of electrical measurements and experimental design of teflon chamber. The H+-selective and reference electrodes (i.e., E_r and E_{H^+}) were connected to the input of differential amplifier (A_3) via two independent probes (A_1 and A_2). Marker of protoplasm streaming cessation is not shown

proved entirely non-toxic for plasmodium vein fragments, which allowed to carry on observations for several hours without visible changes in contractile activity or protoplasm behaviour (shuttle streaming). An example of typical pH oscillations after inserting H^+-sensitive electrode in the drop is presented in Fig. 3. The mean oscillation time of extracellular pH at room temperature is 87 to 114 min (n = 16). Under stable external conditions, the pH oscillation period is a constant even during observations lasting for several hours, whereas different fragments of the investigated veins have their own individual oscillation

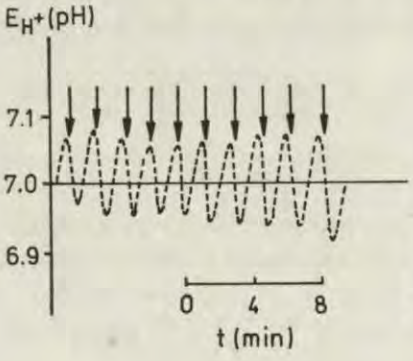

Fig. 3. Typical oscillations of the proton activity (pH) in water droplet deposited on *Physarum* vein. Arrows mark the moment of protoplasm streaming cessation. Temperature 19°C

frequencies. The most stable pH oscillations occurred in the drops of medium situated half-way along the vein fragment. When the drops were situated at the ends of vein fragments, oscillations showed considerable disturbances. During the registration of pH changes in the external medium, the protoplasm behaviour was also under observation and the moments of flow direction changes were marked on the strip chart of a pen recorder. In most vein fragments under investigation there was some correlation between external pH oscillations and the moments of cessation of the protoplasm streaming. However, the experimental methods employed here are not adequate to define with precision the phasic dependences between those two phenomena. That problem will be studied at a later period.

The amplitude of pH oscillations in the drop is mainly dependent on the size of the drop situated on the vein surface. Here the amplitude was from 6 to 18 mV, (i.e., from 0.1 to 0.2 pH, the drop volume being about 0.3 µl).

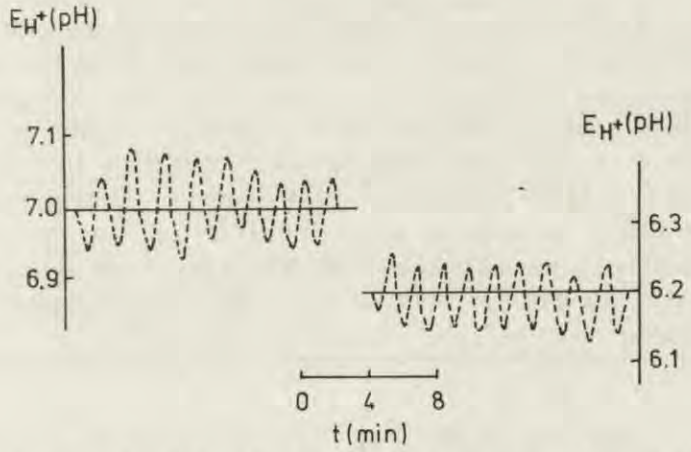

Fig. 4. Illustration of relative displacement of average value of "droplet" pH oscillation to lower pH after different time incubation of fragmented *Physarum* vein. Temperature 19.5°C

Figure 4 compares two oscillation recordings from the same vein fragment, but made at different times. The first recording was taken after 10 min adaptation of the vein, the second after 80 min from the start of the experiment. As can be seen, in both cases there are no essential differences in the nature of oscillations, but mean pH value of the medium decreased by about 0.8 pH unit. Some plasmodium vein fragments showed, under the same conditions, a still higher rate of medium acidification.

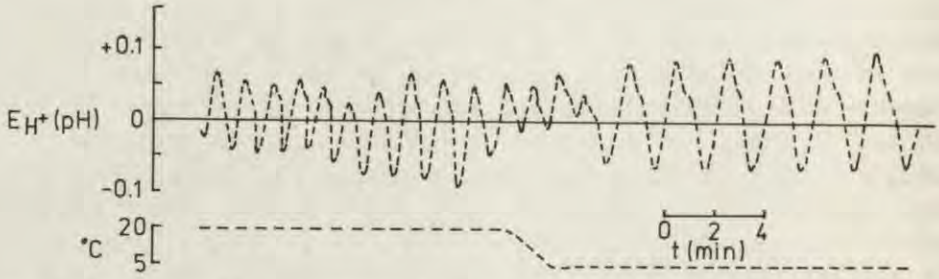

Fig. 5. The proton level oscillation in the water droplet at different environment temperature (i.e., 6°C and 20°C)

A change in ambient temperature of the vein brings about immediate changes in the kinetics of both processes, i.e., oscillation and mean pH decrease. Figure 5 shows one of the typical courses of pH oscillation when ambient temperature changed from 20°C to 6°C. Following the stabilization of temperature at 6°C the observed pH oscillations continue to be stable, as in the case of room temperature, but at a lower temperature the amplitude and time course are extended. Maintaining the veins in that temperature for a longer time slows down the medium acidification rate. At a temperature near 3°C the acidification process ceases entirely. The described effects are totally reversible if the changes of ambient temperature are lower than 10°C/min. Temperature changes have a similar effect on vein contraction activity and protoplasm flows. A drop in temperature is followed by a lowered vein contraction frequency, a lowered protoplasm flow speed and, consequently, the extension of time intervals between subsequent flow direction changes.

Discussion

Studies on plasmodium *Physarum polycephalum* that have been hitherto conducted show that such physiological phenomena as vein contraction activity and protoplasm activity are accompanied, in a more or less pronounced manner, by bioelectrical phenomena. The first studies on that problem suggested the existence of a close cause- and effect-correlation between bioelectrical changes and the rhythm of the driving force activities controlling the so-called "shuttle streaming" of the protoplasm (Kamiya and Abe 1950). Further research, however, failed to confirm these correlations, since electric oscillations have been observed in the absence of protoplasm movement (Kokina and Jiga-

dlo 1964, Meyer and Stockem 1979, Rhea 1966). Electrophysiological experiments on microplasmodia of *Physarum polycephalum* showed that electric oscillations disappear, if surrounding medium is in a continuous flow (Fingerle and Gradman 1982). Therefore, basing on these findings, it has been assumed that electric oscillations are a secondary phenomenon and occur in consequence of processes controlling various manifestations of physiological activity in *Physarum polycephalum*. This seems the more likely as recently there has been observed a close dependence of the veins' contraction activity, as well as of electrochemical ion activity inside these veins, on the metabolic mechanisms of *Physarum polycephalum* (Korohoda et al. 1983, Layrand 1968, Satoh et al. 1982, Wohlfarth-Bottermann 1979).

The results presented in this paper, showing that under definite conditions there occurs an oscillatory release of protons to the medium surrounding plasmodium (Fig. 3, 4) being temperature dependent process (Fig. 5), confirm the possibility that H^+-ion oscillations in the medium are derivatives of the organism's metabolic activity, similarly to the proton dependent generation of resting membrane potential in microplasmodia and plasmodial drops of *Physarum polycephalum* (Fingerle and Gradman 1982, Kuroda and Kuroda 1981). Oscillations and acidification of water drops placed on plasmodium vein fragments are presumably a reflexion of the phenomena observed by Matveeva and co-workers (1977) on entire cultures of *Physarum polycephalum*, which, maintained in weakly buffered media, caused their acidification to the pH value equalling 4.8 to 4.2. The time course of the acidification as well as its magnitude were reproducible for the same culture. As was the case with small drops of medium described in this paper, the lowering of temperature for the whole plasmodia leads to the lowering of medium acidification rate, and with further cooling of the preparation to 2°C the acidification process declines entirely. Possibly, the latter fact reflects an inhibition in the functioning of metabolic mechanisms controlling vein contractility and/or protoplasm movement, or the generation of resting membrane potential (Fingerle and Gradman 1982, Kuroda and Kuroda 1981, also our own unpublished observations).

In conclusion it seems quite likely that electric fluctuations repeatedly observed on the plasmatic membrane of *Physarum polycephalum* are a result of the movement of protons through the plasmodium membrane, which produces ion changes in the medium. The periodic local accumulation of H^+-ions at the external membrane surface also can periodically modify its electrochemical properties, leading to membrane potential fluctuations (Nakamura et al. 1982, Ridgway and

Durham 1976). The introduction of a continuous medium exchange will obviously cause a decline of such a process and, consequently, the electric fluctuations could not be measured.

In the light of the facts confirming the existence of bioelectric changes on protoplasm membrane (Meyer and Stockem 1979, Rhea 1966) without the presence of shuttle streaming in *Physarum*, the bioelectric activity does not seem to result from the distribution of the protoplasm; more likely, there is a direct correlation of electric fluctuation with metabolism. The assumptions expressed in this paper, as well as the possibility of participation of other ions e.g., Ca^{2+} (Teplov et al. 1973, Yoshimoto et al. 1981) in the bioelectric phenomena, require further studies.

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ACTA PROTOZOOLOGICA
Vol. 24, No. 2, pp. 135-138 (1985)

Influence of Biogenic Amines (Histamine, Serotonin) on the Function of the Lysosomal Enzymes of the *Tetrahymena*

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Received on 9 August 1984

Synopsis. Histamine and serotonin, which stimulate the phagocytosis of the *Tetrahymena* to a similar degree, acted differently on the lysosomal phosphatase activity. Since it was shown that histamine stimulated, whereas serotonin depressed it. The lysosomal enzyme inhibitor chloroquine did not in itself depress the phosphatase activity of the *Tetrahymena*, but prevented its stimulation by histamine. The influence of the two hormones on lipase activity was dissimilar.

The unicellular *Tetrahymena*, although it does not by nature possess a hormonal system, it may respond to exogenous hormones, including those of higher organisms, and can even synthesize these. Thus the *Tetrahymena* is able to bind histamine, triiodothyronine, insulin, thyrotropic hormone, follicle stimulating hormone, etc. and can interact with part of these and with certain other hormones (Csaba 1980, 1981, Csaba et al. 1977, Csaba and Ubornyák 1979). The *Tetrahymena* cells have been shown to contain biogenic animes (Blum 1967, Janakivedi et al. 1973), as well as histamine, somatostatin, adrenocorticotropic hormone and beta endorphine (Le Roith et al. 1983).

Histamine acts as phagocytosis-stimulating hormone at the vertebrate level (Jancso 1955). It acts similary on the amoeba (Csaba et al. 1985), and on the *Tetrahymena*, while histamine and serotonin equally act as specific stimulators of phagocytosis (Csaba and Lantos 1973). Since incorporation of extracellular materials represents only the first step of endocytosis, it seemed worthwhile to examine,

Supported by the Scientific Research Council, Ministry of Health, Hungary, 16/1-04, 432/4.

whether or not histamine would also influence the action of lysosomal enzymes. It was expected that this could present evidence on the synchronism or divergence in the hormonal control of the various stages of phagocytosis.

Materials and Methods

Two-day cultures of *Tetrahymena pyriformis* GL cells, grown in 0.1% yeast extract containing 1% Bacto trypton medium (Difco, Michigan, USA) were used. The concentration of hormones administered to cells in normally nourished or starved state (for 24 h in Losina-Losinsky solution) were 10^{-2}, 10^{-3}, 10^{-7} M in the case of histamine (histaminum hydrochloricum, Reanal, Budapest) and 10^{-3} M in the case of serotonin (serotonin creatinine sulphate, Fluka, Buchs, Switzerland). The treatment always lasted 10 min. When necessary the lysosomal enzyme inhibitor, chloroquine (chloroquinum phosphoricum, Egyt, Budapest) was added to the culture at the concentration of 2×10^{-4} M for 24 h.

Phosphatase activity was assayed by the method of Gomori (cit. in Bancroft and Stevens 1982). The incubation time was 5 min, and Na-fluoride was used as inactivating agent at the concentration of 0.01 M/ml, for 20 min at 37°C. The lipase assay was performed as described by Gomori (cit. in Bancroft and Stevens 1982), the incubation time was 4 h.

The phosphatase assay was rendered quantitative by grading the activity found into eight categories, from 0 to complete saturation of the cell, depending on the amount of lysosomes giving a positive reaction. In every case, 500 cells were examined for category distribution; the count found was multiplied by the category number, and the result was divided by 500 to obtain the mean value. These values are registered in Table 1.

The microscopic pictures of differently treated cells are shown in Pl. I-IV.

Table 1

Action of endocytosis influencing hormones on the lysosomal phosphatase activity of the *Tetrahymena*

Group	Nourished (values ± S.D.)	Starved (values ± S.D.)
Control	4.3±0.51	2.6±0.71
Histamine, 10^{-2} M	5.9±0.30	3.9±0.48
Histamine, 10^{-3} M	5.5±0.25	4.0±0.55
Histamine, 10^{-7} M	3.8±0.50	2.2±0.99
Serotonin, 10^{-3} M	2.3±0.94	1.2±1.17
Chloroquine, 2×10^{-4} M	—	3.1±1.01
Chloroquine + histamine, 10^{-3} M	—	2.3±0.83

Numerical data represent the categories from 0 to complete saturation of the cell, depending on the amount of lysosomes giving a positive reaction (Gomori test).

Results and Discussion

As shown earlier (Csaba and Lantos 1973), both histamine and serotonin stimulate the Chinese ink phagocytosis of the *Tetrahymena* to a considerable degree already at a very low concentration (10^{-9} M). The present experiments have suggested that histamine also enhances the lysosomal phosphatase activity of the *Tetrahymena*, although certainly at much higher concentrations. In the presence of 10^{-7} M histamine there was in fact no appreciable difference between the phosphatase activities of the control and the histamine-treated cells, whereas in the presence of 10^{-3} and 10^{-2} M histamine there was a considerable increase over the control. The fact that the serotonin-treated cells showed a marked decrease in phosphatase activity — to about 50% relative to the control — indicated that serotonin enhances only the incorporation of extracellular materials but not the number of the enzyme carrying lysosomes. The hormone effect did not differ between the well nourished and starved cells (Pl. I and II). Nevertheless, there is no explanation, why serotonin decreased the number of phosphatase containing lysosomes.

The lysosomotropic enzyme-inhibitor chloroquine (Poole et al. 1982, Brown et al. 1983) seemed to be indifferent in itself, but supressed entirely the histamine-induced stimulation of phagocytosis (Pl. III).

The comparison of the results of the present experiments and previous studies (Csaba and Lantos 1973, Csaba et al. 1982) have indicated that the trend of hormonal influence on endocytosis may be either identical or different in the successive stages, depending on the nature of the hormone. The fact that the action of histamine was identical (stimulating) to the Chinese ink incorporation (in earlier experiments) and to the number of phosphatase containing lysosomes (data from the present paper) indicates that histamine is possibly a genuine endocytosis hormone.

Chloroquine developed no inhibitory action on the lysosome number in itself, but it suppressed completely the action of histamine; starvation did in itself account for depression of enzyme action.

We failed to elaborate a quantitative technique for lipase assay. The experimental results nevertheless indicate a considerable depression of lipase activity in the conditions of starvation. Histamine depressed lipase activity in normally nourished cells, whereas it enhanced it rather in starved cells. Serotonin stimulated lipase activity in both normally nourished and starved cells (Pl. IV). These observations disclose

no information on the functional context, but support the hypothetical conclusion that the different lysosomal enzymes are dissimilarly affected by hormonal influence.

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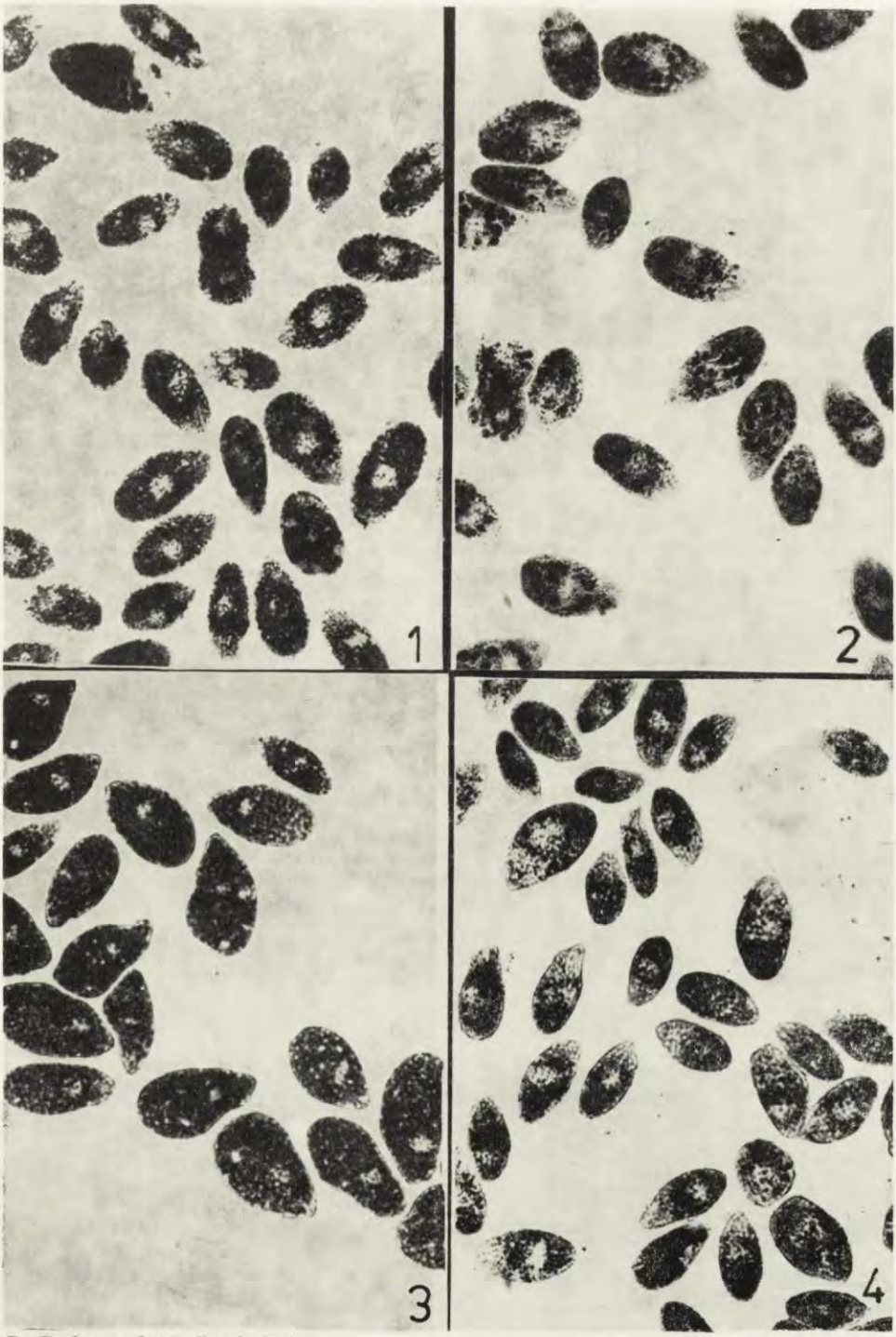
EXPLANATION OF PLATES I-IV

I: Phosphatase enzyme reaction in *Tetrahymena*. × 250, 1 — control, 2 — histamine (10^{-7} M) treated, 3 — histamine (10^{-3} M) treated, 4 — serotonin (10^{-3} M) treated

II: Phosphatase enzyme reaction in *Tetrahymena*. × 250, 1 — control (starved), 2 — histamine (10^{-3} M, starved) treated, 3 — serotonin (10^{-3} M, starved) treated

III: Phosphatase enzyme reaction in *Tetrahymena*. × 250, 1 — chloroquine (2×10^{-4} M, starved), 2 — chloroquine + histamine treated (10^{-3} M, starved), 3 — 0.01 M NaF-treated

IV: Lipase enzyme reaction in *Tetrahymena*. × 250, 1 — control, 2 — histamine (10^{-3} M), 3 — serotonin (10^{-3} M)



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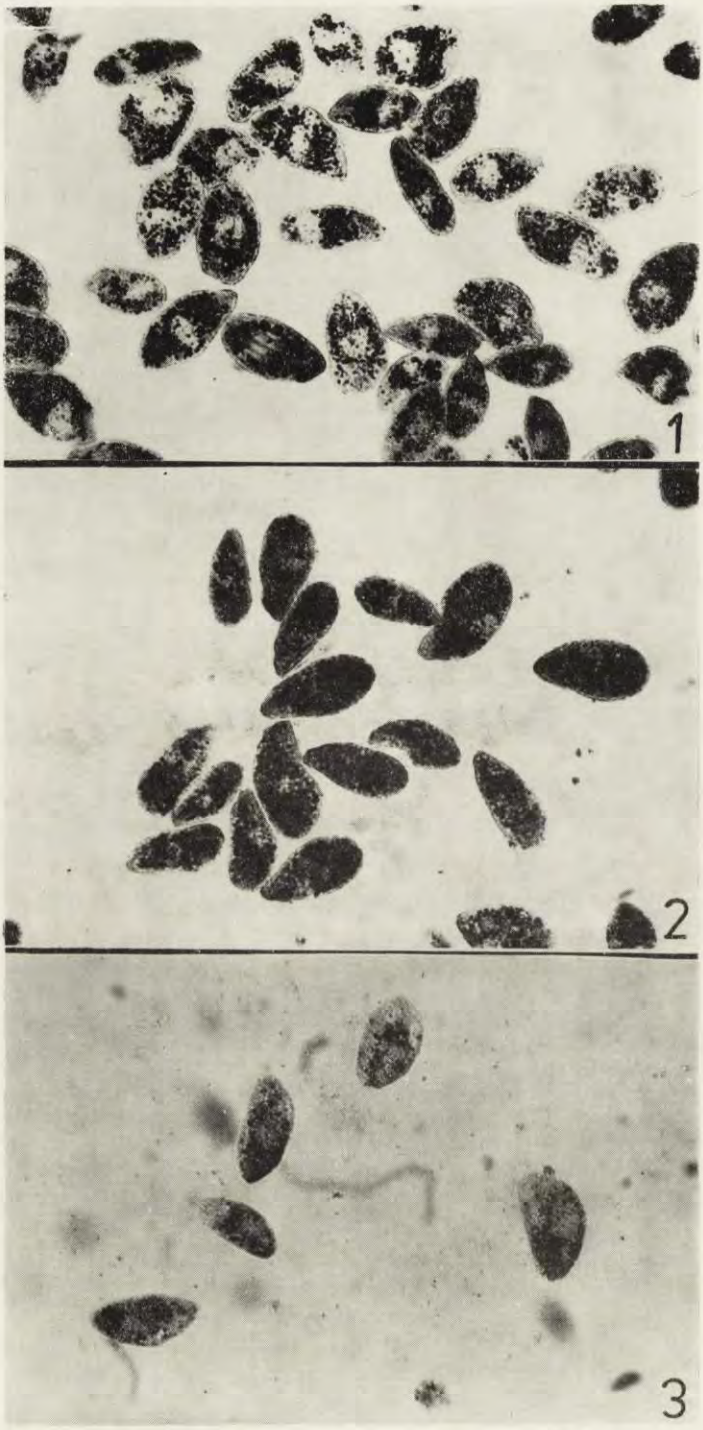

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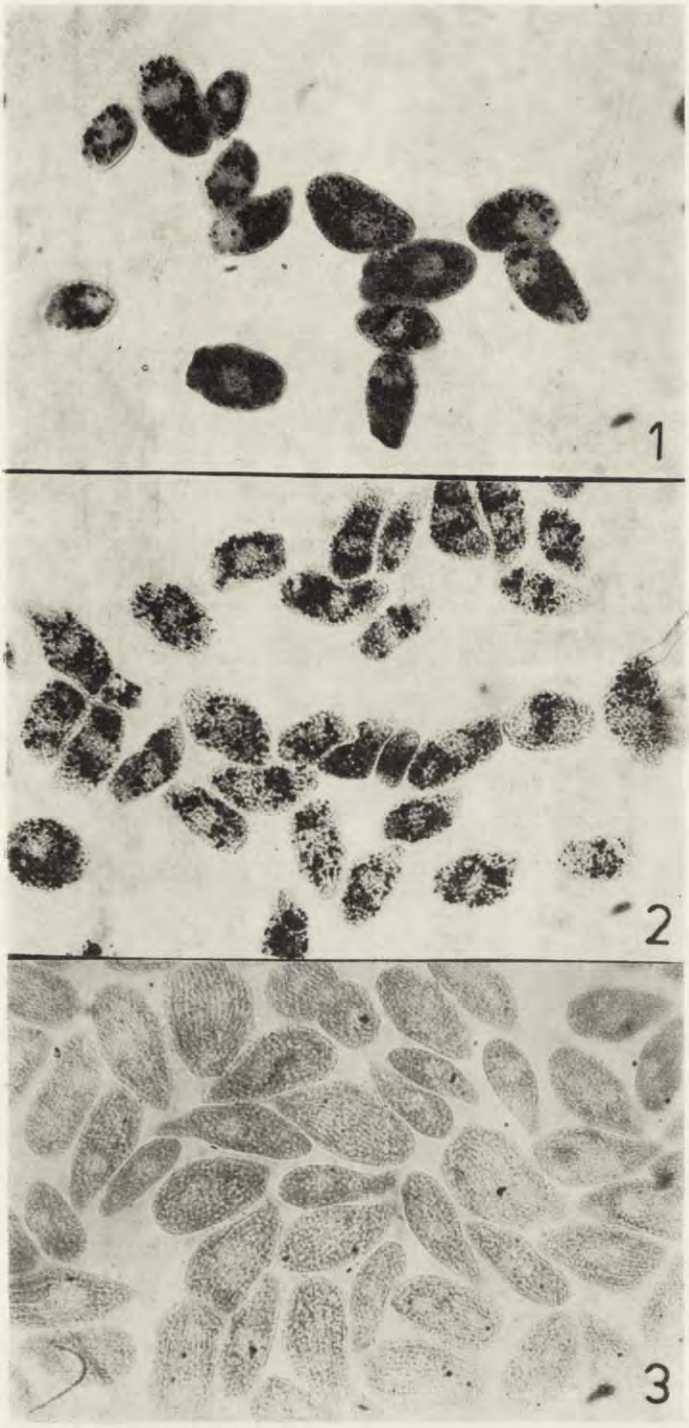

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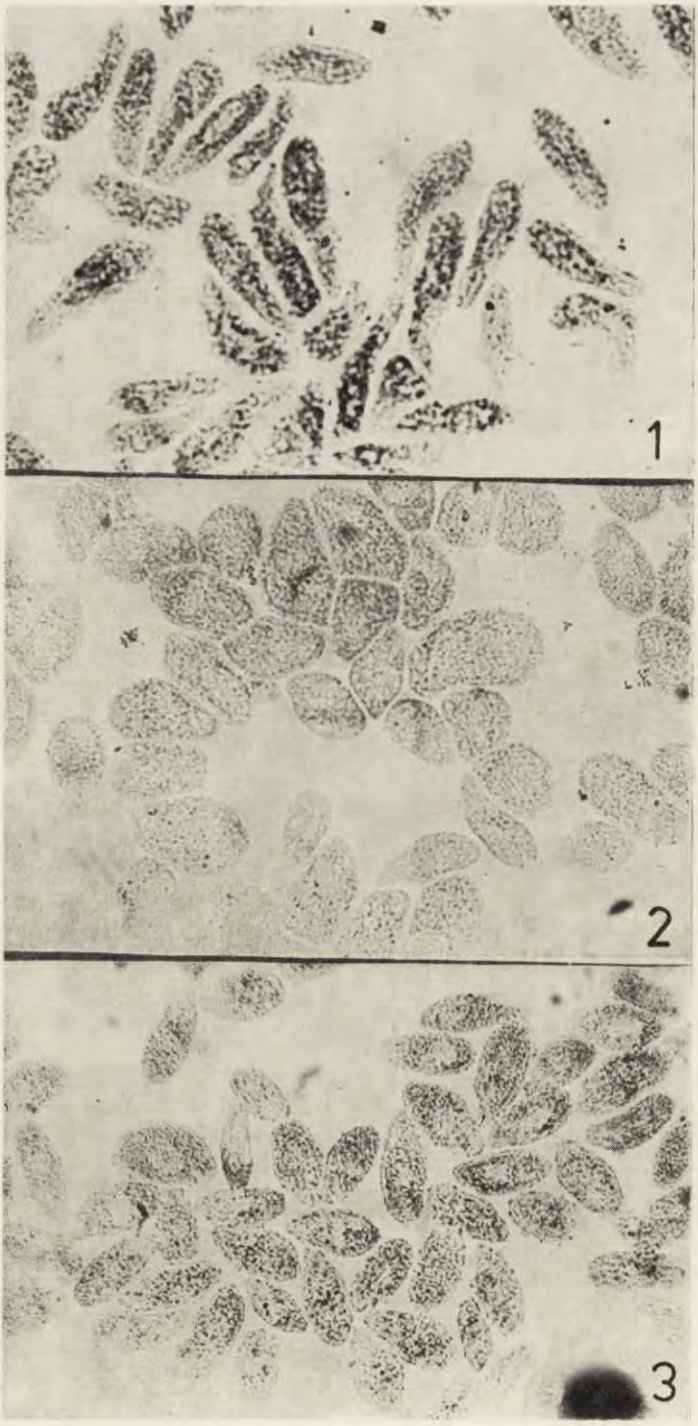

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ACTA PROTOZOOLOGICA
Vol. 24, No. 2, pp. 139-146 (1985)

Tetrameridionospinispora karnataki gen. nov., sp. nov. A New Cephaline Gregarine from Damselfly, *Agriocnemis* sp.

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Received on 30 August 1984

Synopsis. This paper deals with the morphology and life history of a cephaline gregarine *Tetrameridionospinispora karnataki* gen. nov., sp. nov. from an odonate, *Agriocnemis* sp. Ascertained on biconical sporocysts with rows of polar and meridional spines, the parasite has been assigned to a new genus *Tetrameridionospinispora* of the subfamily *Acanthosporinae* Léger emend Grassé. The parasite has a globular epimerite placed on a short neck with numerous tentacles at its periphery and biconical sporocysts with four polar spines, two at each pole and four meridional spines, two on each side. The taxonomic position of *Ancyrophora ceriagrioni* (Nazeer Ahmed and Narasimhamurti 1979) is reviewed in the light of the new generic characters and new combination is proposed with a name *Tetrameridionospinispora ceriagrioni* (Nazeer Ahmed and Narasimhamurti 1979) comb. nov.

In India, although much work has been done on the study of cephaline gregarines, reports of gregarines from odonates are scanty (Sarkar 1981, Sarkar and Haldar 1980, 1981 a-e, Nazeer Ahmed and Narasimhamurti 1979, Narasimhamurti and Nazeer Ahmed 1980 and Kori and Amoji 1983, 1984). It is obvious from the literature survey that some of the odonates have been found to be infested with cephaline gregarines belonging to the subfamily *Acanthosporinae* Léger emend Grassé of the family *Actinocephalidae* Léger. Subfamily *Acanthosporinae* comprises about 17 genera (Levine 1979). Of these, the genera *Acanthospora* Léger

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(1892), *Ancyrophora* Léger (1892), *Cometoides* Labbé (1899), *Ramicephalus* Obata (1953) and *Mukundaella* Sarkar (1981) have biconical sporocysts with polar and meridional spines. Except the genus *Cometoides*, the remaining four genera have been reported from odonate hosts.

During our investigation on cephaline gregarines of odonates from Gulbarga (Karnataka, India) we came across an actinocephalid gregarine. The details of its morphology, life history, percentage of infection and the seasonal intensity revealed that the gregarine has independent generic status in the subfamily *Acanthosporinae* Léger emend Grassé. Affinities of this new genus with the closely related genera in the subfamily are also discussed.

Material and Methods

Odonate insects were collected around Gulbarga University Campus, Gulbarga (Karnataka, India) and on the same day were examined for gregarine infection. The methods followed for the preparation of smear slides, staining and histological techniques employed are similar to those reported earlier (Kori and Amoji 1984).

Various morphometric measurements and the india ink drawings in this paper are made with the aid of camera lucida.

Observations and Discussion

On an average about 21% of insects are found infected. The infection prevails only during September to December months. Formation of gametocysts occurs during December. The examination of histological sections of the infected region of the gut did not reveal intracellular developmental stages of gregarine.

Description of the Species

The smear preparation of the gut content revealed the following various life history stages of the gregarine:

Trophozoites: Trophozoites (Fig. 1 A and B) measuring 168-400 μm in length and 25-100 μm in width are found attached all along the length of the epithelial layer of the midgut wall of the host. The trophozoites, being three-segmented structures, bear a fusiform deutomerite (313.5 μm × 58.13 μm), broadest at the anterior region and gra-

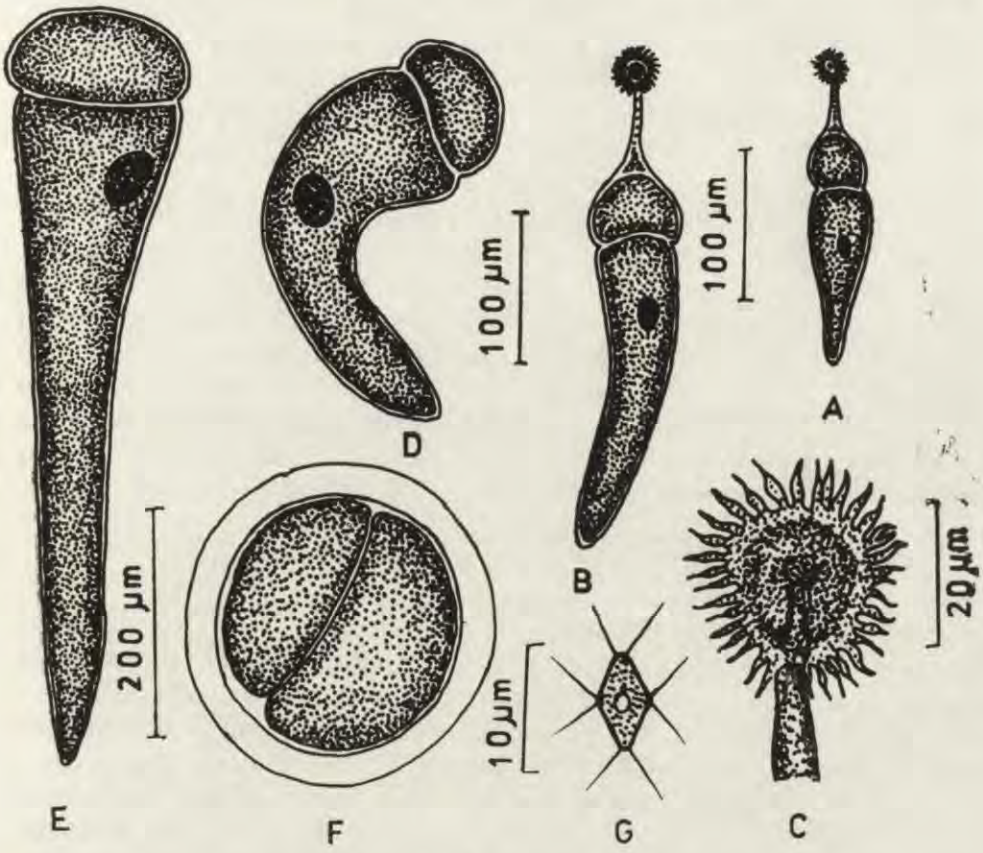

Fig. 1. A-G — *Tetrameridionospinispora karnataki* gen. nov., sp. nov., A — Early trophozoite, B — Later trophozoite, C — Epimerite of the Fig. 1 B enlarged, D-E — Sporadins in various developmental stages, F — Gametocyst, G — Biconical sporocyst with four polar spines and four meridional spines

dually tapering at the posterior extremity. An ovoidal nucleus (35 µm × 19.50 µm) is enclosed in the granular endoplasm of the deutomerite. The protomerite (51.8 µm × 55.0 µm) is dome-shaped. The epimerite (Fig. 1 C), measuring 62.25 µm in length, bears a globular or discoidal knob (25 µm × 25 µm) at the apex of a short (29.15 µm) neck. The periphery of the knob (Fig. 1C) is drawn into numerous (34-39) sharp-tipped tentacles.

Sporadins: Sporadins (Fig. 1 D, E) are solitary, with a fusiform body measuring 125-715 µm in length and 30-200 µm in width. The protomerite is subspherical (55.63 µm × 93.44 µm) measuring relatively more in width than in length. Its width is nearly same as that of the deutomerite (PW : DW, 1 : 0.88-1.25). The length of the protomerite is about four to eight times shorter than that of the total length

of the sporadins (PL : TL, 1 : 4.0-8.3). The deutomerite increases its length as the development progresses and it gradually tapers into a conical tip at its posterior end. It measures 266.25 μm in length and 96.56 μm in breadth. An oval nucleus (35.83 μm × 21.9 μm) is enclosed in a dense granular endoplasm of the deutomerite.

Gametocysts: The gametocysts (Fig. 1F) are spherical, milky-white bodies with a thin (10 μm) cyst wall enveloped within a smooth uniformly thick (25 μm) ectocyst. The gametocysts after 8-10 days of their development in a moist chamber mature and release sporocysts dehiscing by simple rupture of the cyst wall.

Sporocysts: The sporocysts are refractile biconical structures (Fig. 1 G). They measure 7 μm × 4 μm. Each sporocyst bears four polar spines (two et each pole) and four meridional spines (two on each side).

Systematic Position

The features of the presently described gregarine such as: sporadins solitary, epimerite globular or discoidal with numerous sharp-tipped tentacles at its periphery and biconical sporocysts with polar and equatorial spines, confirm its inclusion under the subfamily *Acanthosporinae* Léger emend Grassé of the family *Actinocephalidae* Léger.

The subfamily *Acanthosporinae* includes, among others, the genera *Acanthospora*, *Ancyrophora*, *Ramicephalus*, *Mukundaella* and *Cometoides*, all of which are characterized by the presence of biconical sporocysts with polar and equatorial spines, the latter being six in number.

Although the above genera resemble each other closely in the nature of their sporocysts, they vary markedly in their structure of epimerite i.e., simple conical knob in *Acanthospora*, globular or discoidal with tentacles or spines in *Ancyrophora*, dendroidal disc in *Ramicephalus*, cup with vertical undulations on its wall in *Mukundaella* and globular with long slender filaments directed upwards in *Cometoides*. The epimerite of the presently described gregarine resembles to some extent that of the genus *Ancyrophora* in being globular or discoidal with numerous tentacles or digitiform processes at its periphery. But the genus *Ancyrophora* is quite distinct, as it is characterized, like other four genera, by the presence of six spines at an equator of the biconical sporocysts as against four in the new genus. The comparative features of the genera bearing sporocysts with polar and meridional spines under the subfamily *Acanthosporinae* Léger emend Grassé showing the distinctiveness of the genus *Tetrameridionospinispora* gen. nov. are given in Table 1.

Table 1

Comparative features of the different genera having biconical sporocyst with polar and meridional spines

Genera	Characters	
	Epimerite	Sporocyst
Acanthospora Léger, 1892	Non-appendiculate, simple knob	Biconical with one row of polar and one row of equatorial spines, the latter being six in number
Ancyrophora Léger, 1892	Globular, five to twelve backwardly directed digitiform processes	Biconical, with one row of polar and one row of meridional spines, the latter being six in number
Cometoides Labbé, 1899	Globular, six to eight long filaments directed upwards	Cylindrobiconical with one row of polar and two rows of meridional spines
Ramicephalus Obata, 1953	Discoid with many upwardly directed dendroidal processes.	Biconical with one row of polar and one row of equatorial spines, the latter being six in number
Mukundaella Sarkar, 1981	Broad, wide cup with numerous closely-set vertical undulations on a short neck	Diamond-shaped, hexagonal in polar view, two polar and six meridional spines
Tetrameridionospinispora gen. nov.	Globular or discoidal with numerous tentacles or spines	Biconical, with two polar spines at each pole and two equatorial spines on each side

Diagnosis

The diagnostic features of the *Tetrameridionospinispora* gen. nov. are as listed below:

(1) Sporadins solitary.

(2) Epimerite placed on a short neck is globular or discoidal with numerous peripheral tentacles having pointed tips.

(3) Gametocysts with ectocyst dehisce by simple rupture.

(4) Sporocysts biconical with four polar spines (two at each pole) and four meridional spines (two on each side).

(5) Gut parasites of odonate insects.

Species: *Tetrameridionospinispora karnataki* gen. nov., sp. nov.
Host: *Agriocnemis* sp.
Habitat: Midgut region
Locality: Gulbarga University, Gulbarga, Karnataka, India
Repository: Department of P. G. Studies and Research in Zoology, Gulbarga University, Gulbarga, Karnataka, India

Tetrameridionospinispora ceriagrioni (Nazeer Ahmed and Narasimhamurti 1979) comb. nov.

During their study on septate gregarines of the damselfly, *Ceriagrioni coromandelianum* from different localities in Visakhapatnum, Andhra Pradesh, India, Nazeer Ahmed and Narasimhamurti (1979) reported a gregarine *Ancyrophora ceriagrioni* with the following diagnostic features:

(1) Cephalonts elongate, cylindrical, having epimerite in the form of shallow cup, the margin of which is produced into 17-20 digitiform processes.

(2) Sporonts solitary.

(3) Cysts spherical, 255-350 μm in diameter with an ectocyst 40 μm thick, dehiscing by simple rupture.

(4) Spores biconical with four polar spines, two at each pole and four equatorial spines, two on each side.

Nazeer Ahmed and Narasimhamurti (1979) considered the features of this species identical with the characters of the genus *Ancyrophora* Léger and named it *Ancyrophora ceriagrioni.*

It is obvious from the description of the species that the features of the various stages of the gregarine are not in conformity with those of the genus *Ancyrophora.* The flexible epimerite may reveal various shapes due to changes in fixation and fixatives used. The cup described by the authors may be due to the lateral view of the sunken state of the disc-like epimerite.

The sporocysts described by the authors are with four polar and four equatorial spines, exactly resembling the sporocysts of the type species described for the genus *Tetrameridionospinispora*. Moreover, the sporocysts being the resistant stage in the life history of gregarine, will have its structure unchangeable for a given species.

Hence, the characters of the *Ancyrophora ceriagrioni* Nazeer Ahmed et Narasimhamurti are more in conformity with the genus *Tetrameridionospinispora*. Therefore, a new taxonomic status, *Tetrameridionospinispora ceriagrioni* (Nazeer Ahmed et Narasimhamurti) comb. nov., is proposed for this gregarine species.

ACKNOWLEDGEMENTS

We express our deep indebtedness to the Professor and Head of the Department of P. G. Studies and Research in Zoology, Gulbarga University, Gulbarga, for the necessary facilities extended to carry out this work and to Dr. S. J. Brooks, CAB identification Service, Commonwealth Institute of Entomology, British Museum (Natural History), London SW7 5BD, England, for the help in identifying host specimens.

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ACTA PROTOZOOLOGICA
Vol. 24, No. 2, pp. 147-152 (1985)

Babesia bengalensis sp. n. (*Apicomplexa: Babesiidae*), a New Piroplasmid from a Common Indian Mongoose, *Herpestes edwardsi* (Geoffroy)

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Received on 13 February 1984 and *revised on* 24 September 1984

Synopsis. A new piroplasmid, *Babesia bengalensis* has been described from a common Indian Mongoose, *Herpestes edwardsi* (Geoffroy). The young forms are either oval or piriform measuring 2×1.5 μm with an average area of 2 μm². The mature trophozoites are mostly piriform measuring 3.5×1.8 μm with an average area of 4.5 μm². The trophozoites multiply by means of binary fission and schizogony. The product of schizogony are tetrads which arranged either cross-wise or in the form of a fan. The relationship of *B. bengalensis* with the other *Babesia* spp. from mongoose has been discussed.

Babes (1888) in Roumania was the first to describe *Babesia* parasites in the blood of cattle and sheep. Subsequently Smith and Kilborne (1893) demonstrated the tick transmission of *Babesia bigemina*, the causative agent of bovine babesiosis. Since then, 71 distinct species of *Babesia* have been reported from various vertebrate hosts until today (Levine 1971). *Babesia* are intraerythrocytic parasites structurally very similar to protozoa (Ristic 1970). Unlike *Plasmodium*, however, *Babesia* does not leave residual hemozoin pigment following ingestion of food vacuole-stored haemoglobin. There have been reported from different parts of the world at least six *Babesia* species in various mongoose, viz. *Babesia legeri* (Bedier 1924), *B. herpestedis* (Franca 1908), *B. cynicti* (Neitz 1938), *B. vanhoofi* (deSmet and Lips 1955), *B. heschi* Grewal, 1957 and *B. hoarei* Grewal, 1957. (*Vide* Ristic and Lewis 1977).

In India, Patton (1910) for the first time reported one *Babesia* sp. from *Herpestes mungo* in Madras, but he neither named the pa-

rasite nor described it in detail. Manwell and Kuntz (1964) described one *Babesia* from Indian Bandicoot, *Bandicota indica nemorivaga*. The present paper deals with a new species of *Babesia* from *Herpestes edwardsi*, which constitutes a new host-parasite record from this part of Indian subcontinent.

Material and Methods

The host specimens were collected from Barasat, 24-Parganas district, West Bengal, India. Out of five hosts examined only one was found infected. The intensity of the infection was moderate to high. The infected host was dead. The blood smears were drawn from the blood directly taken from the heart during post-mortem examination. Imprints of different organs viz. liver, lung, spleen etc. were also taken. Blood smears and organ imprints were fixed in 100% methanol, stained with Romanowsky's stains and observed under high resolution microscope. Camera-lucida drawings were made on graph paper of mm division facilitating area measurements. Measurements were taken with the help of an occular micrometer. Some photomicrographs were taken with the help of Olymphus PM6 camera.

Observations

Babesia bengalensis sp. n.
Fig. 1 A–L, Pl. I 1–4)

Host: *Herpestes edwardsi* (Geoffroy)
Locality: Barasat, 24-Parganas district, West Bengal, India
Site of Infection: Blood
Vector: Unknown

Description: These are intraerythrocytic parasites round, oval or piriform in shape. The young forms (Fig. 1 A, B) measure 2 μm by 1.5 μm with an average area of 2 μm^2 (N = 15). These are mostly piriform (Pl. I 1). Sometimes sickle-shaped forms are also found (Fig. 1 C, Pl. I 2). The cytoplasm is very clear. Nucleus is in the form of a chromatin dot either placed centrally or peripherally. Sometimes the nucleus is insignificant, occupying the circumference of the prasite.

The mature trophozoites are mostly piriform measuring 3.5 by .8 μm with an area of 4.5 μm^2 (N = 15). Some broad forms (Fig. 1 D, E) measuring 3.7 by 2.0 μm are also observed. Their nuclei are in the form of chromatin dots located marginally and sometimes minute vacuoles are also noted. The trophozoites multiply by means of binary fission and schizogony. The product of binary fission are pairs, either

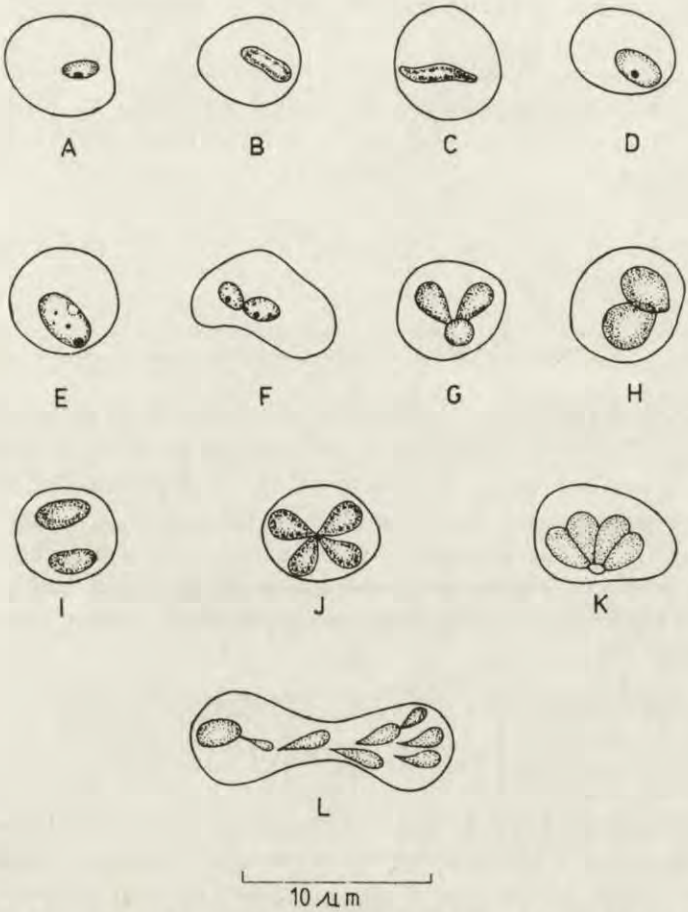

Fig. 1 *A-L*. Camera-lucida drawings of *Babesia bengalensis* from Indian Mongoose, *Herpestes edwardsi*, *A-C* — young forms of *B. bengalensis* sp. n., *D, E* — the mature trophozoites, *F-H* — the schizonts with closely attached merozoites, *I* — two merozoites being separated, *J* — the cross-wise arrangement of merozoites, *K* — the merozoites arranged side by side, *L* — hypertrophied erythrocyte containing eight merozoites, the products of two schizonts

closely attached (Fif. 1 *F–H*, Pl. I 3) or separated (Fig. 1 *I*, Pl. I 4). Each merozoite is round in shape, measuring 2.2 µm in diameter with an average area of 5 µm² (N = 15). The product of schizogony are tetrads (four merozoites). They are arranged either cross-wise (Fig. 1 *J*) or side by side in the form of a fan within the erythrocyte (Fig. 1 *K*). Each merozoite of the tetrad is piriform, measuring 2.5 by 1.8 µm with an average area of 3.5 µm².

Sometimes a single erythrocyte contains eight small piriform merozoites (Fig. 1 *L*), which seems to be derived from the schizogony of two trophozoites.

Effect of the Parasite on Host Erythrocytes

The infected erythrocytes became hypertrophied in length but atrophied in width. They measure 6.6 by 4.7 μm with an average area of 24.9 μm^2 (N = 10). In case of double infection the erythrocytes became very much hypertrophied in length showing dumble-like (Fig. 1 *L*) in appearance.

Normal erythrocyte: N = 10. Cell measures 5.8 × 5.4 μm and 24.9 μm^2 in average area.

Diagnosis of *Babesia bengalensis* sp. n.

These are intraerythrocytic parasites. The young forms are piriform measuring 2 μm by 1.5 μm with an average area of 2 μm^2. The cytoplasm is clear. Nucleus is in the form of a chromatin dot either placed centrally or peripherally. The mature trophozoites are mostly piriform measuring 3.5 by 1.8 μm with a mean area of 4.5 μm^2. The trophozoites multiply both by means of binary fission arranged either cross-wise or in the form of a fan within the erythrocyte. Each merozoite measures 2.5 by 1.8 μm.

Discussion

A thorough review of the literature revealed that there are many reports of various *Babesia* sp. in. wild and laboratory adapted mammals throughout the world. Unfortunately, many of these reports are only incomplete bits and pieces based on unconfirmed observations. Most of these parasites were given separate specific status due to their occurrence in different hosts. However, their structural similarity apts the modern knowledge that there is relatively little host specificity in the *Babesiidae*. While discussing the taxonomy of the piroplasms, Levine (1971) stated that extreme caution should be taken for naming new pirosplasms solely on the ground that they are found in new hosts. He also suggested that 23 of the 71 species of *Babesia* which he listed may actually be synonyms of *B. quadrigemina* (Nicolle 1907) or *B. muris* (Fantham 1905). But cross-transmission experiment is very much needed before taking such action.

The present species has got certain resemblances with the young form of *B. bandicootia* Manwell and Kuntz, 1964, in having pear-shaped appearance with small chromatin dots, but differs greatly by not possessing tenous and extremely long pseudopodia in trophozoites. The

schizonts of *B. bengalensis* sp. n. deviate from *B. bandicootia* by showing clear tetrad (four merozoites) arranged cross-wise or in the form of a fan, which is absent in the latter.

The young forms of *B. heischi* Grewal, 1957 and *B. hoarei* Grewal, 1957 have some similarities with the present parasite in the structure and general configuration. But the trophozoites or adult forms of the before mentioned species appear circular, ring-like, resembling a young malaria parasite, whereas the trophozoites of the present species are mostly piriform and never stretching across the red cells. The characteristic cross forms, which are uncommon in *B. heischi* and *B. hoarei*, are quite common in the present species. The chromatin bodies of *B. heischi* and *B. hoarei* divided irregularly (unequal division), whereas the chromatin body of the present species divided equally. Multiple infections are quite common in the former species, but rare in the latter. The species under report differs considerably in size and in other particulars from all other *Babesia* species reported from mongoose.

Therefore, a new name *Babesia bengalensis* sp. n. has been coined for the parasite due to its occurrence in West Bengal, India.

ACKNOWLEDGEMENTS

The authors are very much grateful to Dr. B. K. Tikader, Director, Zoological Survey of India, for providing laboratory facilities. They are also indebted to Prof. B. Dasgupta, Principal, Darjeeling Govt. College, West Bengal for his kind confirmation of the parasite and many suggestions during the work.

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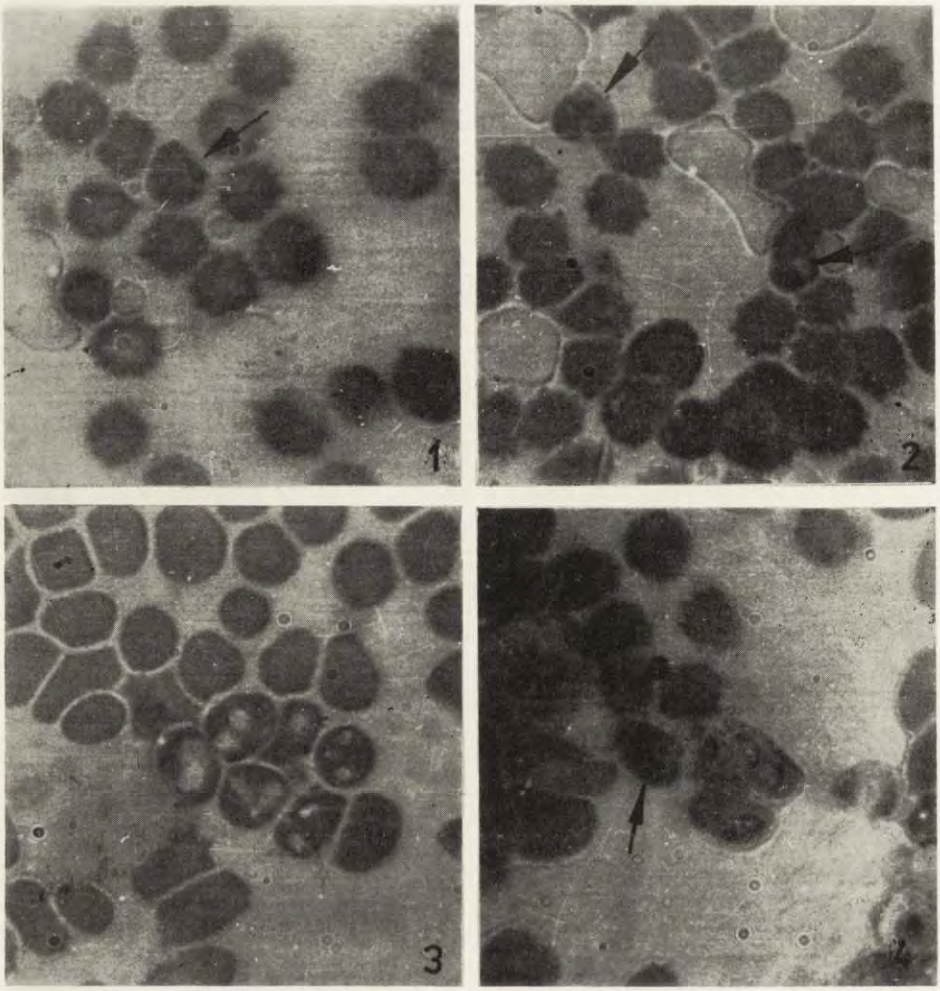
EXPLANATION OF PLATE I

1-4: Photomicrographs of different stages of *Babesia bengalensis* in the erythrocyte of Indian mongoose, *Herpestes edwardsi*

1, 2: Arrow shows the young piriform stage of *B. bengalensis* in the R. B. C. of *Herpestes edwardsi*, ×1360

3: A number of mature schizonts with closely attached merozoites ×1360

4: A number of parasitized erythrocytes showing the schizonts where merozoites are separated; the arrow indicates a schizont with cross-wise merozoites (4), ×1360



S. Bandyopadhyay and R. Ray auctores phot.

ACTA PROTOZOOLOGICA
Vol. 24, No. 2, pp. 153-164 (1985)

A New Microsporidian *Glugea malabaricii* sp. n. from the Viscera of *Carangoides malabaricus* Bl.

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Received on 23 August 1984

Synopsis. The morphology and life history of *Glugea malabaricii* sp. n. from the marine fish, *Carangoides malabaricus* Bl. was described and its systematic position is discussed. Cell hypertrophied tumours or xenomas were seen in the liver, intestine and gonads, but no external manifestations of infection were observed. Merogonic stages in the hypertrophied host cells were observed to have destroyed the host cell nuclei. Sporogonic stages were seen in the sporogony vacuoles in the peripheral zone of the cyst or xenoma.

Microsporidian parasites of fishes have attracted the attention of the parasitologists in the past decade and numerous species have been reported from different parts of the world. From Indian sub-continent, however, there are only a few reports. During the survey of the food fishes of Waltair coast, Bay of Bengal, a new species of *Glugea* parasitizing the liver and intestine of *Carangoides malabaricus* Bl. was recorded. For reasons discussed elsewhere in the paper, it is considered new to science and the name *Glugea malabaricii* sp. n. is proposed for it.

Material and Methods

Carangoides malabaricus Bl. is a common food fish available in the local market throughout the year. They were collected from two different areas which are about 8 km apart in Visakhapatnam; from the net catches at Lawson's

[1] Part of the thesis approved for the award of the Degree of Doctor of Philosophy of the Andhra University, Waltair.

Bay where country craft are used and also from the catches of the Offshore Fishing Station where mechanized boats and modern craft are used for fishing.

The fish were dissected as soon as they were brought to the laboratory to detect infection, since there were no external indications of infection. Different internal organs were examined and since xenomas, when present, were fairly big, they could be identified easily with the unaided eye. Smears prepared from the xenomas of different sizes were either air-dried and stained with Giemsa after an initial hydrolysis in 1 N HCL for 10 min, or wet-fixed in Schaudinn's or Carnoy's fluid and stained with various histological stains. Smears were fixed in alcoholic Bouin's fluid and treated according to the PAS technique to demonstrate the polar cap. Some of the smears were also stained using Feulgen's technique to study the structure and nature of the nucleus.

Observations

Parasite: *Glugea malabaricii* sp. n.
Host: *Carangoides malabaricus* Bl.
Site of infection: Liver, intestine and gonad
Locality: Visakhapatnam, Bay of Bengal (East Coast of India)
Type slides: Author's collections and Dept. of Zoology, Andhra University

Morphology and Life Cycle

There are no external indications of infection except for a slightly bloated up appearence of the abdomen in the heavily infected fish. Large xenomas measuring up to 1 cm (0.5–1.0 cm) were found embedded in the liver tissue. A maximum number of four xenomas were collected from a single fish. When the number of xenomas was more than one, their size was small. Occasionally the xenomas were also seen attached to the ascending limb of the intestine and to the gonad. Cysts attached to the wall of the intestine were smaller in size, measuring 1.0-1.5 mm, but contained mature spores. However, as stated earlier, the cysts in the liver, which is the common site of infection, were larger in size. The cysts could be separated from the surrounding tissue very easily with a pair of needles, which showed that there were no rigid adhesions between the cysts and the host tissue.

The *Glugea* "cysts" were enclosed in a thick, milky white envelope measuring 12-15 μm. They were deeply embedded and surrounded by a cellular layer which showed numerous kupfer cells intermingled with a few wandering phagocytes. The cells were rhomboidal in shape, with their nuclei undergoing amitotic divisions. There is a non-cellular area immediately below the cellular layer. Surrounding the "xenocyte" is

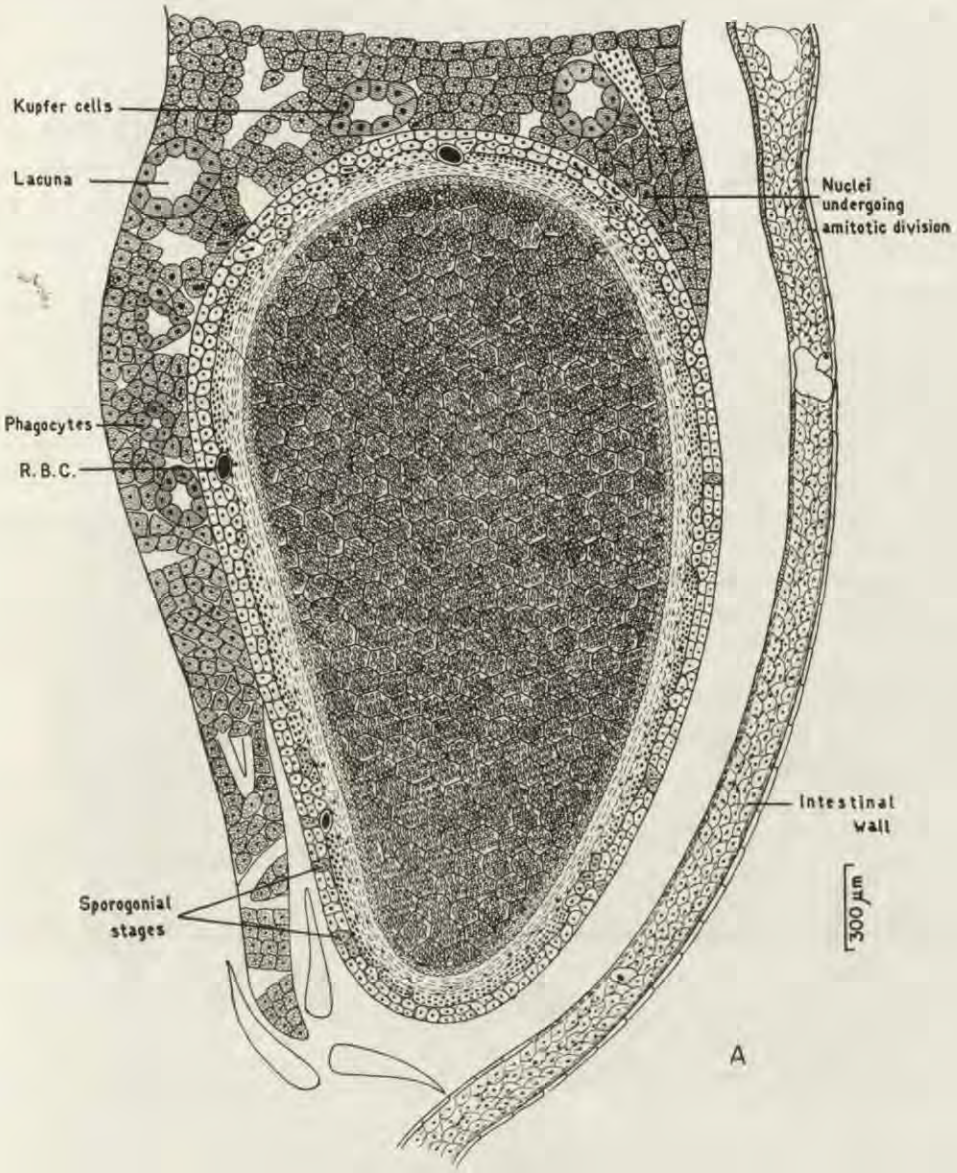

Fig. 1. *A* — Section of the liver showing the cyst

a layer of librous tissue. The xenocyte is a hypertrophied host cell with nuclear fragments distributed in the cytoplasm (Fig. 1 *A*).

The spores of the microsporidian are concentrated in small compartments with a wall.

Stages of merogony could not be followed in detail. But occasionally some stages were seen in the cellular layer. The earliest stage

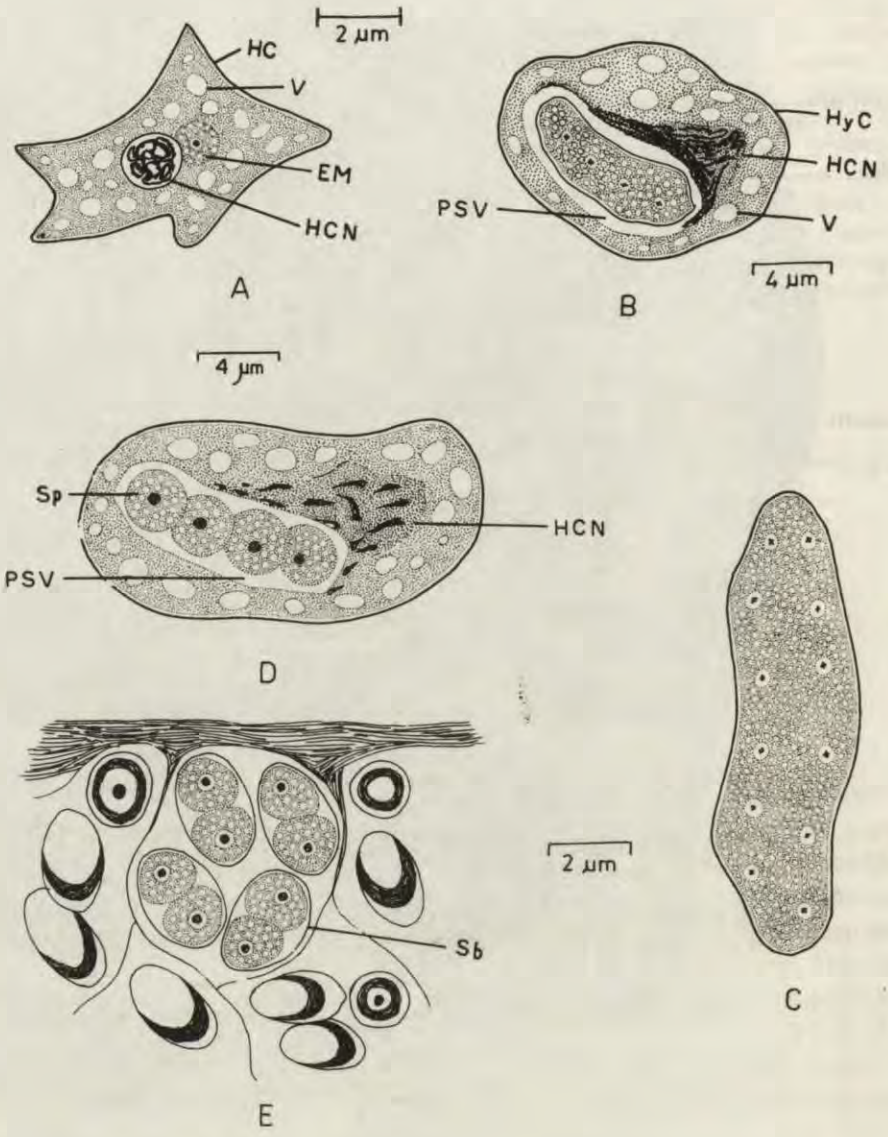

Fig. 2 *A* — A uninucleate meront in the cytoplasm of the hypertrophied liver cell, *B* — A tetranucleate stage, *C* — A multinucleate stage, *D* — Uninucleate bodies arranged in a linear row, *E* — Oval sporoblasts in pairs in compartments of the cyst

Key to lettering: EM — Early Meront, HC — Host Cell, HCN — Host Cell Nucleus, HyC — Hypertrophied Cell, PSV — Parasitophorous Vacuole, Sb — Sporoblast, Sp — Sporont, V — Vacuole

observed in the merogony was oval, measuring 1.0 × 1.2 μm (Fig. 2 *A*) and was found in the host cell cytoplasm, lying adjacent to the nucleus with reticulate chromatin. The nucleus was dot-like and was located in the centre of the pale blue cytoplasm. The host cell was stellate in shape, containing vacuolated cytoplasm. They grew into multinucleate cylindrical plasmodia varying in size from 6.4-9.0 μm × 2.4-3.0 μm and were present in a parasitophorous vacuole. A maximum number of 12 nuclei were seen in these stages (Fig. 2 *B*, *C*). Further stages of division and development could not be followed. The next stage observed was uninucleate bodies arranged in a linear row, probably the "sporoblast mother cells" or sporonts (Fig. 2 *D*). In sections these bodies were always present in a parasitophorous vacuole. Occasionally some of these stages could also be seen in smears prepared from very small xenoma. The sporoblast mother cells were oval or spherical in shape with a large chromatin component. These sporonts eventually divide and form a group of sporonts and each gave rise to two sporoblasts (Fig. 2 *E*).

Oval sporoblasts, sometimes in pairs and sometimes in larger groups, were seen near the periphery of the cyst. While mature isolated spores entangled in a fine layer of mucus were seen in the lumen of the cyst.

Spores

The spores measuring 4.8-5.5 μm × 2.4-3.2 μm were oval with blunt, rounded ends and an outer thick refringent wall. They generally occur in pairs in fresh preparations (Fig. 3 *A*) but they separated off into individual spores either by drying or after fixation. A spherical polaroplast could be clearly seen in the fresh condition at the anterior end (Fig. 3 *B*). Spores stained with Giemsa after an initial hydrolysis in 1 N HCl at 60°C for 10 min showed the sporoplasm in the form of a girdle at the posterior region. The sporoplasm which was finely alveolated and pale blue showed a single small dot-like nucleus (Fig. 3 *C*). A spherical PAS positive polar cap and a finely coiled polar filament were observed in smears stained according to the PAS technique (Fig. 3 *D*). Some of the spores stained with Heidenhain's iron haematoxylin or treated according to the Feulgen's technique showed the nucleus in the form of two small granules (Fig. 3 *E*, *F*). Most of the mature spores, however, showed only a single mass of chromatin material. The spore wall appeared thick and refringent. The polar filament in 90% of spores was released by the addition of a drop of Hydrogen peroxide to the air dried smears. It was uniformly thin, measuring 80-100 μm (Fig. 3 *G*).

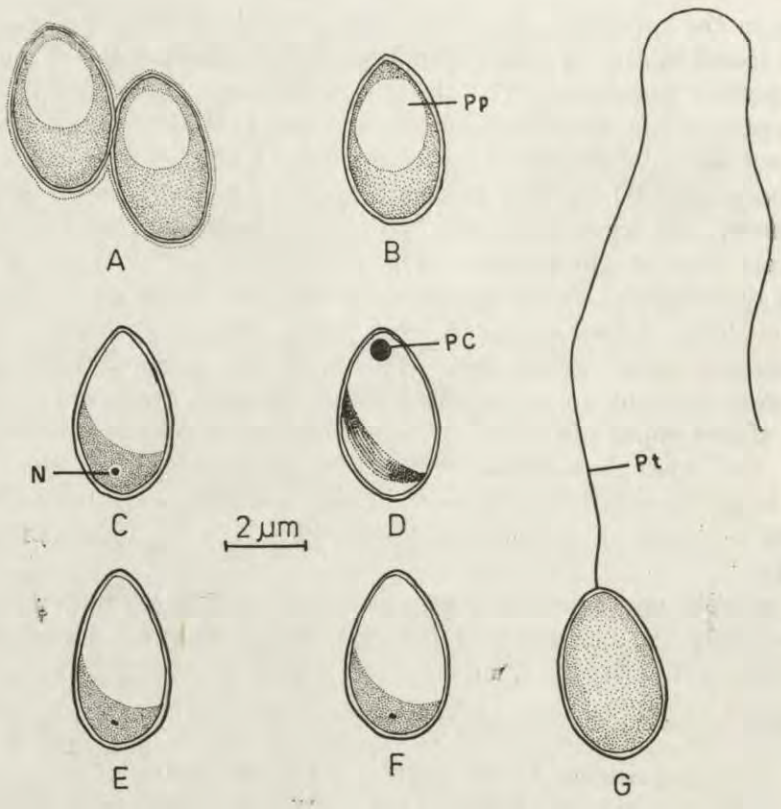

Fig. 3. *A* — A pansporoblast, *B* — Fresh spore, *C* — Spore with dot-like nucleus with the sporoplasm, *D* — Spore stained with PAS technique showing the polar cap, *E* — Spore with dot-like nucleus in the sporoplasm, *F* — Spore stained according to the Feulgen's technique, *G* — Spore showing an extruded polar filament. (For key to lettering see Fig. 2)

Taxonomic Position

The present parasite shows all the characters of the genus *Glugea* Thelohan, 1891 in having a symbiotic xenoma with disporoblastic pansporoblasts and plasmotomy in a parasitophorous vacuole (Voronin 1977).

So far twenty nine species of *Glugea* have been reported from a variety of piscine hosts from different parts of the world (Table 1). A perusal of the Table shows that of the twenty nine species described, so far only five species, *Glugea acerinae* Jirovec, 1930 from the gut wall of *Acerina cernua*, *G. luciopercae* Dogiel and Bychowsky, 1939

from the intestine and mesentery of *Lucioperca lucioperca*, *G. punctifera* Thélohan, 1895 from the muscles of eye of *Gadus pollachius*, *G. tisae* Lom and Weiser, 1969 from the submucosa of the gut of *Silurus glanis* and *G. depressa* Thélohan, 1895 from the liver of *Julis vulgaris* have spores whose measurements range from 3.5-5.0 μm × 1.5-3.0 μm and are comparable with those of the present form. However, in all these cases, the polar filament was long and measured 200-250 μm. In *G. acerinae* the spore has an anterior polaroplast and a posterior vacuole. The xenomas measure 200-300 μm only and have a hypertrophied nucleus. In *G. dogieli* the spores have a pointed anterior end and thus they differ considerably from the present form. In *G. punctifera* a characteristic "highly refringent granule" was reported in the posterior vacuole, which is absent in the present form. The present form differs from *G. tisae* also in having a dot-like nucleus and not a crescent-shaped nucleus. *Glugea depressa* alone is described from the liver and the others are from different sites of infection. The cysts of *G. depressa* were very small, appearing as "white spots" ranging in size from 500-600 μm, whereas in the present form the xenomas reach a maximum diameter of about 1.5 cm.

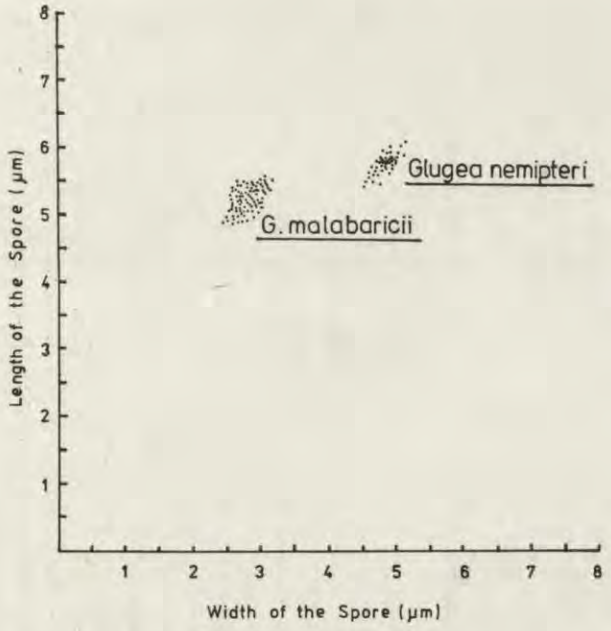

Fig. 4. Scatter diagram showing the size variation in the spores of *Glugea nemipteri* and *G. malabaricii* sp. n.

Table 1

A check list of the species of *Glugea* so far described genus: *Glugea* Thélohan, 1891

Number of species	Name of the parasite, author and year	Host	Site of infection	Spore	Xenoma
1	2	3	4	5	6
(1)	*G. anomala* (= *N. anomala*) Moniez, 1887 Gurley, 1893	*Gasterosteus aculeatus*, *G. pungituis* and *Gobius minutus*	Sub-cutaneous connective tissue, cornea, ovary, peritonium of alimentary canal	3–6×1.5–2 μm Pf. 150 μm	3–4 mm
(2)	*G. acuta* Thélohan, 1895	*Syngnathus acus*, *Entelurus aequoreus*	Connective tissue of the muscle of dorsal fin	5×3–3.5 μm	
(3)	*G. cordis* (= *Nosema cordis*) Thélohan, 1895	*Alosa sardina*	Connective tissue of the heart	3–3.5×2.0 μm	
(4)	*G. depressa* (= *N. depressa*) Thélohan, 1895	*Julis vulgaris*	Liver	4.5–5.0×1.5–2.0 μm	
(5)	*G. punctifera* (= *N. punctifera*) Thélohan, 1895	*Gadus pollachius*	Muscles of the eye	4–5×3.0 μm	
(6)	*G. destreuns* (= *N. destreuns*) Thélohan, 1895	*Callionymus lyra*	Muscles	3–3.5×2–2.5 μm	
(7)	*G. stephani* (= *N. stephani*) (Hagenmuller) Woodcock, 1904	*Pleuronectes platessa* *P. flesus* and *P. americanus*	Muscles, connective tissue, liver and gut	3×1.5 μm	0.5–1.0 mm
(8)	*G. hertwigii* Weissenberg, 1911	*Osmerus eperlanus* *O. mordax*	Intestine, subcutaneous connective tissue	Pyriform 4.6–5×2–3.0 μm Pf. 100 μm	2.0 mm
(9)	*G. branchialis* (= *N. branchialis*) Nemeczek, 1911	*Gadus aeglefinis*	Gill filaments	6.3×3.5 μm Pf. 90 μm	0.2–0.5 mm
(10)	*G. cotti* (= *N. cotti*) Chatton and Courrier, 1923	*Cottus bubalis*	Testis	8–10 μm	700 μm
(11)	*G. acerinae* Jirovec, 1930	*Acerina cernua*	Gut wall	3.5–4.5×2.5–3.0 μm	200–300 μm
(12)	*G. machari* Jirovec, 1934	*Detex vulgaris*	Superficial part of the liver	3.0–4.5×0.8–1.5 μm	300–450×250–280 μm

(13)	*G. luciopercae* Dogiel and Bychowsky, 1939	*Lucioperca lucioperca*	Intestine and mesentary	3.8–5.0×1.8–2.5 μm	91–106 μm
(14)	*G. caulleryi* Van den Berghe, 1940	*Amnodytes lanceolatus*	Liver		
(15)	*G. pseudotumefaciens* Pflugfelder, 1952	*Brachydanio reino*	Ovary, liver, kidney and spleen		
(16)	*G. intestinalis* Chen, 1956	*Mylopharyngodon piceus*	Mucosa of small intestine	6.2×3.6 μm	
(17)	*Glugea* sp. Bogdanova, 1961	*Abramis ballerus*	Wall of the intestine		
(18)	*G. weissenbergi* Sprague and Vernick, 1968	*Apeltes quadracus*	Visceral and parietal peritonium	6.5×3.0 μm Pf. 175 μm	up to 6 mm
(19)	*G. tisae* (= *N. tisae*) Lom and Weiser, 1969	*Silurus glanis*	Submucosa of gut	4–5×2.2–2.6 μm	0.6 mm
(20)	*G. fennica* (= *N. fennica*) Lom and Weiser, 1969	*Silurus glanis*	Subcutaneous tissue fins	6.8–8.1×2.5–3.0 μm	2.5 mm
(21)	*G. dogieli* Gasimagomedov and Issi, 1970	*Lucioperca lucioperca*	Intestinal wall	3.6–4.8×2.4–2.7 μm	200–250 μm
(22)	*Glugea* sp. (Sano, 1970) Putz and McLaughlin, 1970	*Plecoglossus altiveriu*			
(23)	*G. bychowskyi* Gasimagomedov and Issi, 1970	*Alosa kessleri volgensis*	Intestinal wall and testis	3.6–1.8 μm pyriform	
(24)	*G. shulmani* Gasimagomedov and Issi, 1970	*Neogobius caspius* *N. fluviatilis* *N. melanostomus affinis*		2.2–2.4×1.2–1.6 μm	18–80 μm
(25)	*G. gasterostei* Voronin, 1974	*Gasterosteus aculeatus*	Mesenteries	4.9–6.0×2.1–2.8 μm	3.0×4.0 mm
(26)	*Glugea* sp. Awakura, 1976	*Plecoglossus altivelis*		5.9–6.9×2.0–2.4 μm	
(27)	*G. atherinae* Berrebi, 1979	*Atherina boyeri*			
(28)	*G. nemipteri* Weiser et al., 1980	*Nemipterus japonicus*	Gills	5.5–6.0×4.5–5.0 μm	8.0–12.0 mm
(29)	*Glugea* sp. Crandall and Bowser, 1982	*Gambusia affinis*			
(30)	*Glugea malabaricii* (present record)	*Carangoides malabaricus*	Liver, intestine and gonad	4.8–5.5×2.3–3.2 μm Pf. 80–100 μm	up to 1.0 cm

There is only one other report of a microsporidian belonging to the genus *Glugea* from the fishes of India. *Glugea nemipteri* Weiser et al., 1981 is reported from the muscles, gonads and liver of *Nemipterus japonicus* Bl. The parasite reported here differs considerably from *G. nemipteri*, because the spores of *G. nemipteri* measure 5.5-6.0 × 4.5-5.0 μm whereas in the present form they are smaller and measure 4.8-5.5 × 2.4-3.2 μm. The scatter diagram drawn for the spores of both the species clearly shows the size variation in the two species (Fig. 4 *A*). The polar filament in *G. nemipteri* was 40-60 μm, while in the present form it is 80-100 μm. There are few differences in the merogonic sequence also. A maximum number of 12 nuclei are seen in the plasmodia of the present form, unlike to *G. nemipteri* where 16-32 or sometimes even 64 nuclei were observed. Further, the earlier stages in the present form develop in close association with the disrupted host cell nuclei, unlike to *G. nemipteri*.

Of the twenty nine species of *Glugea* listed, eleven were originally described as *Nosema* species. Sprague (1977) in his "Systematics of Microsporidia" transferred them to the genus *Glugea*, as he felt that *Glugea* is mainly a genus of fishes and *Nosema* is that of insects. A detailed description is not available for any of the species and the authors depended mostly on the spore size and the host for identifying the parasites. As suggested by Weiser et al. (1981) "...these cases deserve registration and revision including synonymization from time to time...".

In view of the above mentioned differences and because of the presence of oval spores with broad rounded ends, containing a large polaroplast, its occurrence in a different zoogeographical region, and its presence in *Carangoides malabaricus* only, obviously specific to the host, the present form is considered new to science and is designated as *Glugea malabaricii* sp. n.

ACKNOWLEDGEMENTS

One of us (BVS) is grateful to the Council of Scientific and Industrial Research, New Delhi, for the award of a Junior Research Fellowship and the University Grants Commission, New Delhi for the award of a senior Research Fellowship under the Departmental Support during the tenure of which this work has been completed. We are thankful to Prof. Jaroslav Weiser, Institute of Entomology, Czechoslovak Academy of Sciences, Prague for the many helpful suggestions. Finally we wish to thank the authorities of the Andhra University, for the excellent facilities provided to carry out this work.

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ACTA PROTOZOOLOGICA
Vol. 24, No. 2, pp. 165-174 (1985)

Seasonal Variation of the Microsporidian, *Glugea malabaricii* Parasitic in the Liver of *Carangoides malabaricus* Bl.

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Received on 29 August 1984

Synopsis. The seasonal distribution of *Glugea malabaricii* occurring in the liver of *Carangoides malabaricus* has been studied. The prevalence of infection showed a correlation with the pattern of host biology. Infection was maximum in the winter months (November, December and January), during which the host fish were abundantly available and minimum in summer months (April, May and June), when the availability of the host fish was low.

The seasonal variation and the incidence of infection of the microsporidian parasites of fishes have been attempted by several authors. Stempell (1904), Wissenberg (1911, 1913), Mavor (1915), Schrader (1921), Reichenow (1929), Haley (1953), Stunkard and Lux (1965), Delisle (1969), McVicar (1975) and Takavorian and Cali (1981) studied the intensity and incidence of infections in different size groups of fishes and also from different geographical regions. Haley (1953) observed that the incidence of infection is high and that it was heavy among very young fish. Delisle (1969) observed that there is an increase in the incidence of infection of *Glugea hertwigii* during the months of July, August and September. Mc Vicar (1975 and Olsen (1976) studed the variations in the occurrence of *Glugea stephani* in the lower and upper estuaries.

[1] Part of the Thesis approved for the award of the Doctorate degree of the Andhra University.

In the present study an analysis of the incidence of infection and its seasonal occurrence based on the collections made during the years 1979-1981 has been made with a view to correlate the findings with the host biology.

Material and Methods

Carangoides malabaricus Bl. is a common food fish available in the local market throughout the year. A total number of 1045 fishes were collected from the Offshore Fishing Station at Visakhapatnam during 1979-1981. The fishes ranged in size from 4-20 cm and belonged to both sexes.

The fishes were dissected as soon as they were brought to the laboratory to detect infection, since there were no external indications. Different internal organs were examined and it was found that xenomas, when present, were fairly big and could be identified very easily with the unaided eye. Various histological staining procedures were used to study the structure of the spores and developmental stages from the smears prepared from the xenomas.

Observations

The host fish, *Carangoides malabaricus* occurs throughout the year but was more abundant during October-February period. During this period the fishes were sexually mature (gonads ripe containing 7th stage ova). The breeding season for the host appeared to be from December to March. This being winter on the east coast of India, water

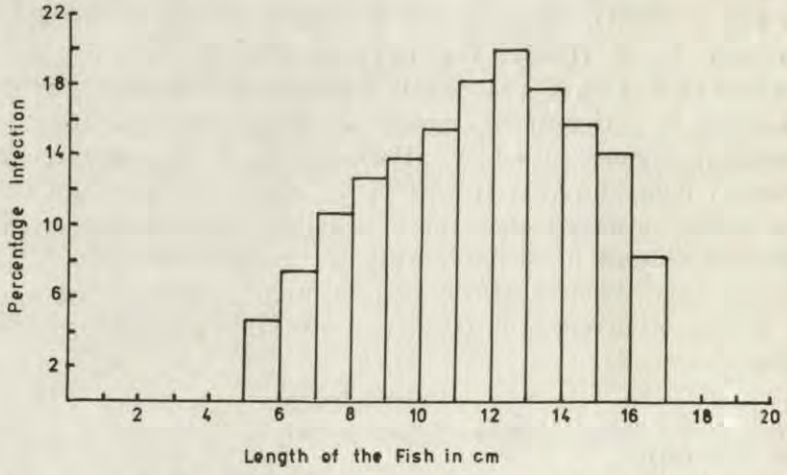

Fig. 1. Incidence of infection in different size groups of *Carangoides malabaricus*

Table 1

Availability of *Carangoides malabaricus* and rate of incidence of *Glugea malabaricii* during 1979–1980 and 1980–1981

	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Total
1979–1980													
Number of fish examined	42	34	35	13	46	48	37	42	51	54	64	54	520
Number of fish infected	7	2	—	—	—	4	5	8	8	11	16	18	79
Percentage of infection	16.66	5.88	—	—	—	8.33	13.51	19.04	15.68	20.37	25.00	33.33	15.19
1980–1981													
Number of fish examined	38	28	36	28	39	56	38	40	46	48	72	56	525
Number of fish infected	8	4	1	—	—	3	3	6	7	12	21	22	87
Percentage of infection	21.05	14.28	2.27	—	—	5.35	7.89	15.00	15.20	25.00	29.16	39.28	16.57

Table 2

Distribution of the *Glugea malabaricii* in different sexes of *Carangoides malabaricus* during 1979–1980 and 1980–1981

Number of fish examined	Number of male fish examined	Number of male fish infected	Percentage of infection	Number of female fish examined	Number of female fish infected	Percentage of infection	Number of Juveniles[1] examined	Number of Juveniles[1] infected	Percentage of infection
1979–1980	183	21	11.47	264	53	20.07	73	5	6.84
520									
1980–1981									
525	176	24	13.63	278	63	22.66	71	—	
Total 1045	359	45	12.53	542	116	21.40	144	5	3.47

[1] Juvenile: Fish in which gonads are not visibly formed are referred to as Juveniles

temperature generally ranges from 18-25°C. Fishes collected during the months of May, June and July were smaller in size and the gonads were not formed. They varied in size from 4-6 cm. There was a progressive increase in the size of the host fish through August, September and October and it reached the maximum size and peak in availability during November and December (Fig. 1).

The percentage infection of *Glugea malabaricii* Narasimhamurti et al., 1985 showed a correlation with the pattern of host biology (Table 1, Fig. 2). The percentage infection reached the maximum in the mature males and females during the breeding season of the fish (November, December, January and February), the temperature ranging between 18-25°C. During the period of two years when the hosts were examined no infection was observed in the summer months of the year (April, May and June, when the temperature ranged between 30–38°C) immediately following the breeding season (Table 2). Infection started again in the month of August and the xenomas were very small and few.

The incidence of infection in different size groups of *Carangoides malabaricus* showed that the percentage infection was higher in the fish ranging in size from 10 to 16 cm. The percentage infection in

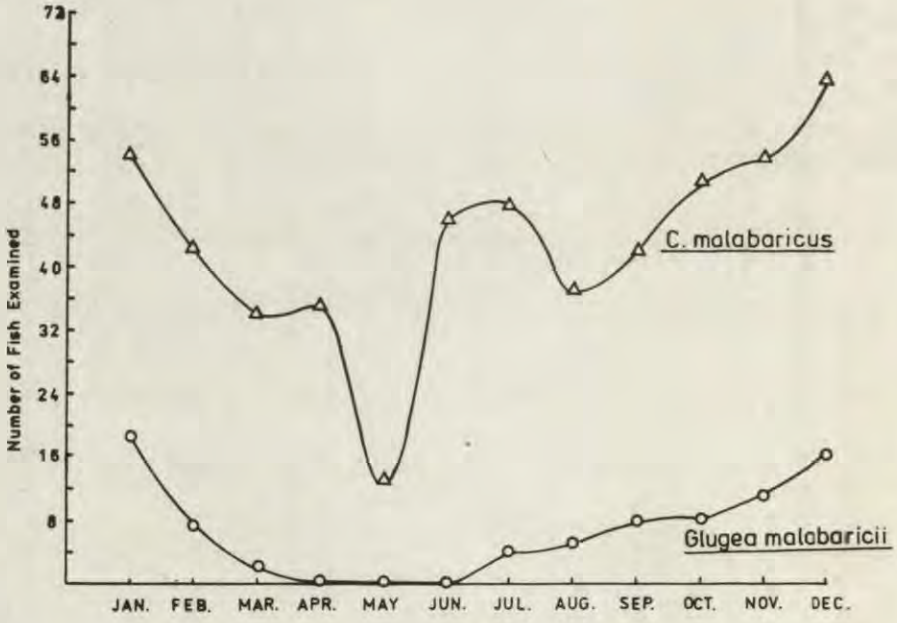

Fig. 2. Availability of *Carangoides malabaricus* and rate of incidence of *Glugea malabaricii* in the year 1979-1980

Table 3

Incidence of *Glugea malabaricii* in different size groups of *Carangoides malabaricus*

Length (in cm)	Number of fish examined	Number of fish infected	Percentage infection
1–2	—	—	—
2–3	—	—	—
3–4	—	—	—
4–5	8	—	—
5–6	22	1	4.54
6–7	54	4	7.40
7–8	56	6	10.71
8–9	63	8	12.69
9–10	72	10	13.86
10–11	102	16	15.68
11–12	207	38	18.35
12–13	268	54	20.14
13–14	101	18	17.82
14–15	44	7	15.90
15–16	21	3	14.28
16–17	12	1	8.33
17–18	5	—	—
18–19	2	—	—
19–20	1	—	—
Total	1045	166	15.885%

smaller fish was less (Table 3). Since the development of such massive xenomas was bound to take some time, it is quite possible that the infection might have started early in life when the fish were smaller in size and passed through different stages of development before conspicuous xenomas could be observed in the liver. The absence of xenomas in the summer months of April, May and June showed that the infection was probably less in fingerlings. The water temperature plays an important role as also the food habits. McVicar (1975) made similar observations in *G. stephani* and demonstrated by the transmission experiments that the parasite depends on high temperature (15-18°C). Similar observations were made by Takavorian and Cali (1981), who found that the peak is in October and the fall in May in the New York-New Jersey complex. It is also possible that the plankton feeding of the fingerlings is a hindrance for the infection. Schrader (1921) also observed that the size of the fish was a criterion for infection.

The above observations show that the apparent infection starts in the fish after they reach a certain size (not in fingerlings) in late summer months (June and July) and develops to form a conspicuous xenoma

by winter (December-January). The temperature of the water mass during these periods varied from 23-30°C. The percentage infection slowly decreased during March-April to reappear again in the month of May. Unfortunately it had not been possible to maintain the fingerlings in the laboratory to carry out experimental infection studies. Hence the observations were entirely based on infections occurring in nature.

In collections made during the years 1979-1980 and 1980-1981 it was observed that females showed a higher percentage of infection, amounting to 21.4%, while in males it was only 12.53%. The smaller fish (juveniles) showed a still lower percentage infection of 3.47% (Table 2, Fig. 3).

A maximum number of four xenomas ranging in size from 1.0-1.5 cm were found in a single fish. Generally xenomas collected from mature

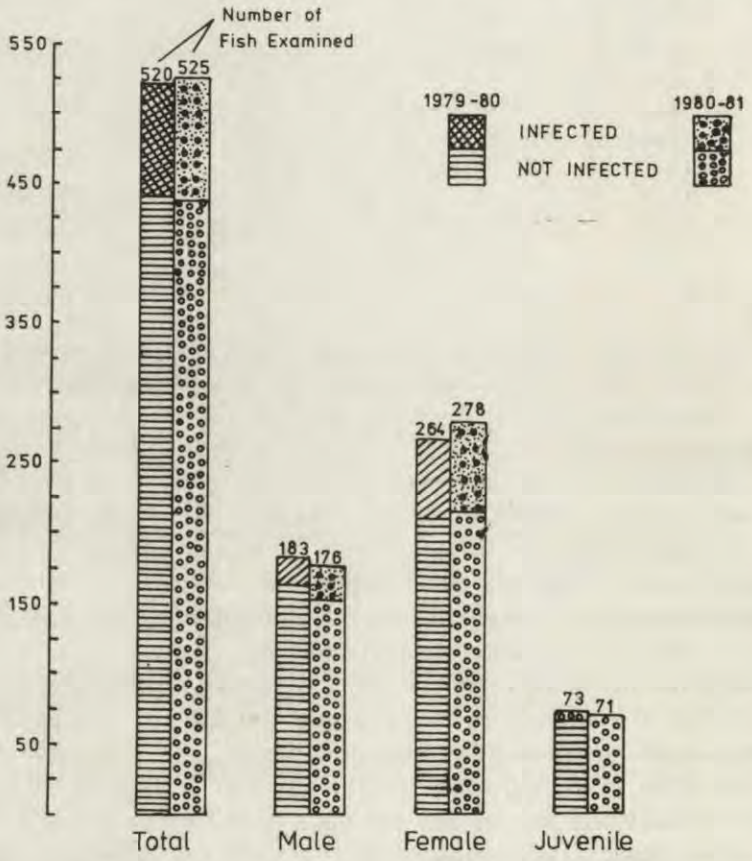

Fig. 3. Distribution of *Glugea malabaricii* in different sexes of *Carangoides malabaricus* during 1979-1981

female fish were bigger in size when compared to those collected from mature male fish, size of the fish being the same. Juveniles when infected showed cysts measuring 2-3 mm in diameter.

The rate of incidence of infection varied in different species of the parasite and also appeared to be dependent on the temperature of the water. Wissenberg (1911) reported that 2% of the smelts in the Baltic sea were infected with *Glugea hertwigii*. Mavor (1915) reported that there is an increase in the percentage infection during summer months in *Glugea stephani* infecting the intestine of *Pseudopleuronectes americanus*.

According to Schrader (1921), 28-53% of the smelt *Osmerus mordax* collected from the lakes in New Hampshire were infected with *G. hertwigii*, but he felt that the size of the fish is also an important factor in the incidence of infection. His observations showed that immature fish measuring about 10 cm showed the highest incidence. Adult fish were rarely infected. It may be possible that they might have acquired some immunity, having been infected once and recovered from infection.

Stunkard and Lux (1965) made very interesting observations on the incidence of infection. According to them there was apparently no effect of the season or sex on the incidence of infection. There was no relationship between infection and the amount of food in the stomach. Further, Stunkard and Lux (1965) stated that fishes heavily infected in the first year did not survive in the second year.

Delisle (1969), while working with *G. hertwigii* and its seasonal incidence, came to the conclusion that the percentage infection increased from a minimum of 6.76% in June to a maximum of 93.2% by the end of September with a steep increase in August and September. He felt that the invasion and the eventual cellular hypertrophy takes at least three months to be established solidly (May 22-August 31) after hatching. August-September are summer months when the temperature of the medium was 21° which was favourable for the growth of the parasite. Haley (1953) suggested that the summer months were favourable for the growth of the adult smelts in New Hampshire also.

Similar observations were made by McVicar (1975), Awakura (1976) and Olsen (1976) in *G. stephani*, where the growth of the xenoma was comparatively faster at a higher temperature of 15-18°C and the growth was more pronounced in the first two months of infection. Juveniles were more likely to be infected.

The observations made in the present study show that the percentage infection was maximum in the winter months of November to

January and minimum in the summer months of April-June. This is probably because of the variations in the temperature. The winter temperatures of the subsurface waters off Waltair coast are usually around 20°C, while in summer months the temperature was around 30°C. Delisle (1969), McVicar (1975) and Olsen (1976) oberved that the temperatures of 21°C and 15-18°C are probably the highest during the year in the temperate countries and occur in summer months, while in tropical countries the corresponding temperatures are in the winter season (around 21°C). This clearly shows that the ideal temperature for microsporidian infections ranged between 18-21°C, but neither lower nor higher. Probably the pattern of life cycle changes to suit these ecological conditions.

The period from May to June, when no apparent infection was observed in the present form, is probably the time taken for the parasite to restart the infection and to develop into visible xenomas, as in the case in *G. hertwigii* (Delisle 1969).

A strange correlation between the growth, maturity of the host and the development rate and incidence of parasites has been observed. The parasites increase in numbers simultaneously with the growth of the host, and by the time the host reaches maturity, it shows fully formed (usually largest) xenomas. The winter temperature apparently being favourable for the host and conductive to the growth of the parasite.

Although there is no considerable evidence to show that the spores are released along with the eggs during spawning season, the occurrence of xenomas of the maximum size and containing mature spores in the mature fishes showing 7th stage ova suggests that this is possible and is probably one of the methods adopted by the parasite for the dispersal of the spores.

Although any specific influence of the sex of the host and the rate of infection cannot be assigned, the observations showed that the percentage of infection was more in females than in males, juveniles being rarely infected. This is in contrast to Stunkard and Lux (1965), who stated that the sex of the host has no apparent effect on the growth of the parasite. Olsen (1976) observed that juveniles were more susceptible to infection than the adult fish. Since the observations are based on the collections made from the Offshore Fishing Station, it has not been possible to adduce any reasons for the difference in infection of the fishes belonging to the two sexes, unless it is substantiated by experimental infection studies. It has been observed that the fingerlings of *Carangoides malabaricus* were less infected than the

mature fish. Since the observations are mainly dependent on the field collections and the presence of xenomas, these are probably the fish that have survived the initial infection. The mortality of some of those fingerlings could have contributed to the drop of infection levels in summer.

ACKNOWLEDGEMENTS

One of the co-authors (BVS) is grateful to the Council of Scientific and Industrial Research for the award of a Senior Research Fellowship during the tenure of which the present work was completed. We are thankful to the authorities of the Andhra University, Waltair for the facilities.

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ACTA PROTOZOOLOGICA
Vol. 24, No. 2, pp. 175-180 (1985)

Some Myxosporida (*Myxozoa: Myxosporea*) of Anabantid Fishes of West Bengal, India

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Received on 18 July 1984

Synopsis. Two coelozoic myxosporida (*Myxozoa*: *Myxosporea*), *Myxidium fasciatum* sp. n. and *Myxosoma trichogasteri* sp. n. are described from the gall bladder of an anabantid fish *Trichogaster fasciatus* Bl. Schn. of West Bengal, India. The characteristic dimension of the spore of *Myxidium fasciatum* sp. n. is 16.0 μm×5.9 μm and that of *Myxosoma trichogasteri* sp. n. is 15.55 μm × 9.35 μm. A myxosporidan *Myxidium leiberkuhni* Butschli, 1881 reported from the Indian anabantid *Anabas testudineus* Bl. by Chakravarty (1943) has been compared with the description given by Kudo (1966) and is considered as a new species for which the name *Myxidium mukundae* sp. n. is proposed.

The anabantid fishes constitute one of the common fresh water food fishes of India and only three species of myxosporida have been reported so far from the members of this family (Chakravarty 1943, Kalavati et al. 1981, Richard et al. 1981). This paper describes two more species of myxosporida from the gall bladder of an anabantid fish *Trichogaster fasciatus* Bl. Schn. and re-examines the taxonomic status of *Myxidium leiberkuhni* Butschli, 1881 reported by Chakravarty (1943) from the gall bladder of an Indian anabantid *Anabas testudineus* Bl.

Material and Methods

All autopsies were made from the fresh fishes collected from the lakes of Barrackpur (about 25 km north of Calcutta). The fresh myxosporidan spores, treated with freshly prepared Lugol's iodine solution and the dry smears of the fresh spores stained with Giemsa after fixa-

tion in absolute Methanol, were examined under the oil immersion lens of the Olympus research microscope. The extrusion of the polar filament was achieved with 1% urea solution. All the measurements were taken in micrometer (μm) and the figures were drawn with the aid of a camera lucida.

Observations

Myxidium fasciatum sp. n.

Description

Vegetative form: These were coelozoic, hyaline, oval (17.6 × 12.8-22.5 × 19.0) to spherical (19.0-21.0), disporus.

Spore: These were coelozoic, cylindrobiconical with pointed extremities; shell valves were smooth and thin-walled; suture was thin and S-shaped (Fig. 1 *A*, *B*). The two polar capsules were pear-shaped in valvular view (Fig. 1 *C*) and oval to spherical in sutural view. Each polar capsule had 2-3 coils of polar filament. The polar filament was short and ribbon-like. The extracapsular space of the spore was filled with finely granular binucleate sporoplasm; iodinophilous vacuole was absent.

Dimensions (based on twenty fresh spores; range is given with the mean within the parenthesis):

Length of spore — 14.4-17.6 (16.00)
Width of spore — 5.6-6.4 (5.90)
Length of polar capsule — 4.0-4.8 (4.50)
Width of polar capsule — 3.2-4.8 (4.00)
Infection locus: Gall bladder
Incidence: Three fish infected out of 12 examined
Host: *Trichogaster fasciatus* Bl. Schn.
Locality: Barrackpore, West Bengal

Remark: The present myxosporidan resembles *Myxidium leiberkuhni* Butschli, 1881, *M. heteropneustesi* Chakravarty, 1943, *M. procerum* var. *calcariferi* Chakravarty, 1943, *M. aor* Lalitakumari, 1969, *M. glossogobi* Chakravarty, 1939 and *M. striatusi* Sarkar, 1982 reported from Indian fishes viz., *Anabus testudineus* Bl., *Heteropneustis fossilies* (Bl.), *Lates calcarifer* (Bl.), *Macrones aor* Ham., *Glossogobius giuris* Ham. and *Channa striatus* Bl. respectively in having same infection locus viz., gall bladder. Among them, *M. heteropneustesi*, *M. glossogobi* and *M. striatusi* resemble the present myxosporidan in having closely related mensural data of their spores. However, the latter species differs from

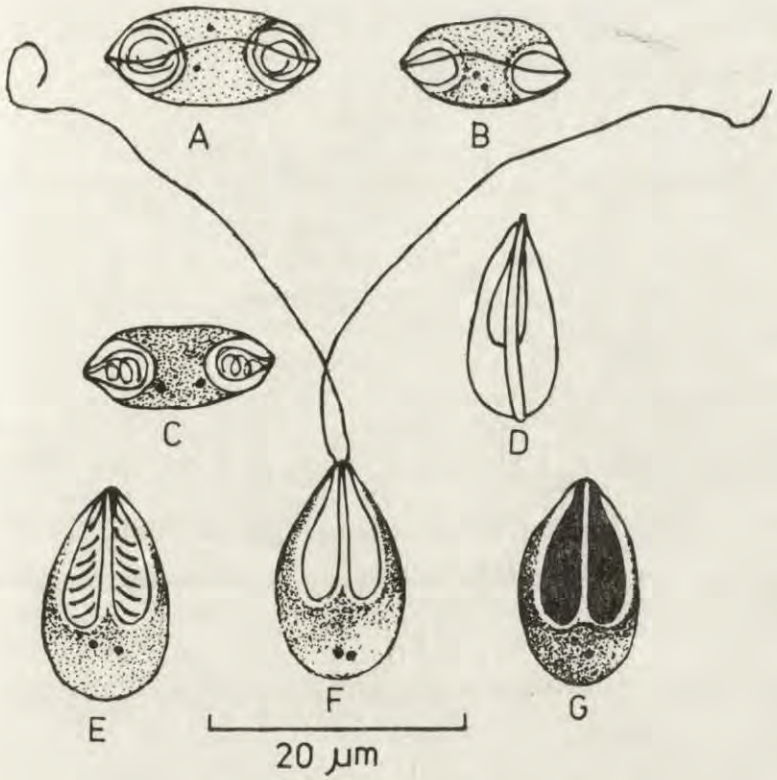

Fig. 1. *A-C*. Spores of *Myxidium fasciatum* sp. n. *A*, *B* — Fresh spores in sutural view — treated with Lugol's iodine solution, *C* — A spore in valvular view — stained with iron alum haematoxylin, *D-G* — Spores of *Myxosoma trichogasteri* sp. n., *D* — A fresh spore in sutural view, *E* — A fresh spore in valvular view — treated with Lugol's iodine solution, *F* — A spore in valvular view with extruded polar filaments — stained with Giemsa, *G* — A spore in valvular view — stained with Giemsa

the former first two species in having cylindrobiconical, smooth spore (spindle-shaped with fine longitudinal striations in *M. heteropneustesi*; elongately oval with rounded extremities in *M. glossogobi*) and also from *M. striatusi* in having smooth shell valve and distinct S-shaped suture (striated shell valve and straight suture in *M. striatusi*). Moreover, the spore of the present species is larger and wider than that of *M. leiberkuhni* Butschli, 1881 (12.4-15.0 × 4.12-5.0) the only *Myxidium* species reported from an Indian anabantid *Anabas testudineus* Bl. (Chakravarty 1943). The myxosporidan in study is, therefore, considered to be a new species and the name *Myxidium fasciatum* sp. n. is given after its host.

Myxosoma trichogasteri sp. n.

Description:

Vegetative form: Not seen.

Spore: The spores were coelozoic, almost lenticular in sutural view (Fig. 1 *D*) and broadly pyriform in valvular view with broadly pointed anterior and rounded posterior end (Fig. 1 *E*). The shell valves were symmetrical, thin-walled and smooth. The suture was thick and slightly bent (Fig. 1 *D*). The two pyriform polar capsules were equal in size, arranged parallel to each other and opened side by side but never crossed (Fig. 1 *E-G*). Each polar capsule had 5-6 coils of polar filament. When extruded the polar filament attained a length of 55.0-60.0 μm (Fig. 1 *F*). The extracapsular space was completely filled with finely granular mass of binucleate sporoplasm. The iodinophilous vacuole was absent in the sporoplasm. Dimensions (based on 25 fresh spores; range is given with mean within the parenthesis):

Length of spore — 14.00-17.00 (15.55)
Width of spore — 8.70-9.85 (9.35)
Length of polar capsule — 9.00-10.47 (10.10)
Width of polar capsule — 3.00-3.84 (3.32)
Infection locus: Gall bladder (bile)
Incidence: Four fish infected out of 12 examined
Host: *Trichogaster fasciatus* Bl. Schn.
Locality: Barrackpore, West Bengal

Remark: The present myxosporidan agrees with *Myxosoma channai* Kalavati et al., 1981 reported from the fins, body muscles, liver and kidney of *Channa punctatus* Bl. in its spore length (14.5-18.0 μm in length of *M. channai*) but disagrees with the latter in having hyaline spore with two equal, pyriform polar capsules (pale brown spore in *M. channai* with two unequal, elongate and pear-shaped polar capsules). It also resembles *M. noblei* Sarkar, 1982 reported from the gall bladder of *Channa striatus* Bl. in having the same infection locus and two equal polar capsules. However, the myxosporidan differs from the latter species by the slightly bent suture and smooth shell valves (S-shaped suture and striated shell valves in *M. noblei*), parallel polar capsules (convergence in *M. noblei*) and larger spore (11.5-14.3 in *M. noblei*). Moreover, the present species is also distinct from *M. magauddi* Richard et al., 1981, the only *Myxosoma* species reported from anabantid fish viz., *Trichogaster fasciatus* Bl. Schn. by the larger dimensions of the spores (10.8-11.7 in *M. magauddi*). Thus, the present myxosporidan is believed to be a new species for which the name *Myxosoma trichogasteri* sp. n. is proposed after the name of its host.

Discussion

So far, only three myxosporidan species (excluding the present work) viz., *Myxidium leiberkuhni* Butschli, 1881 (Chakravarty 1943), *Ceratomyxa* gobiodesi Chakravarty, 1939 and *Myxosoma mugauddi* Richard et al., 1981 have been reported from the gall bladders of *Anabas testudineus* Bl., of *Colisa fasciatus* Bl. Schn. and from the gill filament of *Trichogaster fasciatus* Bl. Schn. respectively (the fishes belong to family *Anabantidae*). Among them, *Myxidium leiberkuhni* Butschli (cited from Kudo, 1966) does not agree in shape with the Indian species reported by Chakravarty (1943), although both have used the term "fusiform". Moreover, Chakravarty's species is much smaller than the original species (12.4-15.0 in Indian species while it is 18.0-20.0 in original species). Furthermore, the Indian species has been reported from the gall bladder, while the original species has been described from the urinary bladder of a very distantly placed fish *Essox lucinus* L. Choudhury and Nandi (1973) supported Chakravarty's view and also reported a new gobiid host *Boleopthalmus boddaerti* Palles for this myxosporidan. However, in view of such distinct differences between the two descriptions, I propose that Chakravarty's species should be considered new to science. The name *Myxidium mukundae* sp. n. is, therefore, given to it after the first name of Prof. Mukunda Murari Chakravarty.

ACKNOWLEDGEMENTS

The author is grateful to Dr. H. Gupta and Sri A. Pramanik, Principal and Head of the Department of Zoology of this College, for laboratory facilities and to the U. G. C. New Delhi for financial support. Thanks are also due to Sri D. Dasgupta of the Department of Zoology, University of Calcutta for his co-operation.

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