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Nuclear DNA Content in *Amoeba proteus* Cultured at Different Temperatures

Received on 27 May 1982

Synopsis. The relative DNA content was measured in nuclei isolated over different intervals after synchronous divisions of *A. proteus* (strain B) using cytofluorometric technique. In amoebae cultured at 25° the nuclear DNA content at the and of interphase exceeded 2.2 times and at 17°, 2.5 times, the corresponding level observed 1 h after division. If amoebae cultured at 17° are transferred immediately after division to 4° (the temperature at which they fail to divide), their nuclear DNA content increases only twice in two weeks of incubation at a low temperature. The absence of extra DNA synthesis in amoebae kept at 4° is due to the effect of low temperature and not to their starvation at this temperature. After the transfer to 25° of amoebae maintained at 4° during a fortnight, the DNA synthesis is resumed in their nuclei and during 48 h (24 h with and 24 h without food) the original doubled DNA content increases up to a level characteristic of the end of interphase in amoebae incubated constantly at 17°. The amount of extra DNA to be synthesized depends on the quantity of food consumed: in starving amoebae (48 h without food) it is lower than in feeding amoebae (48 h with food). At 4° the division is blocked in amoebae with doubled as well as with more than doubled DNA content.

The DNA synthesis during the cell cycle of *A. proteus* has some peculiar features: (1) The cell cycle lacks a G_1 phase, (2) The DNA synthesis starts immediately after division and proceeds during the whole interphase, (3) The nuclear DNA content during interphase increases more than twice (Ord 1968, Ron and Prescott 1969, Minassian and Bell 1976, Chatterjee et al. 1979, Makhlin et al. 1979 a).

Taking into account the peculiarities of the DNA synthesis during the cycle and the specific features of temperature adaptations in *A. proteus* (reviewed in Sopina 1978), our aim was to study the DNA content in the nuclei of these amoebae depending on the temperature regime of their cultivation. Special emphasis was laid on the temperature of 4° at which amoebae do not reproduce (Sopina 1976). This temperature blocks both cytokinesis and karyokinesis (Sopina et al. 1982). But when amoebae after prolonged cultivation at 4° are transferred to a temperature optimal for their reproduction, they remain viable and resume division. Therefore, lengthy survival without division at 4° may be regarded as an adaptive character of these unicellular organisms.

Materials and Methods

Mass cultures or synchronized groups of *A. proteus* (strain B) were cultured according to Prescott and Carrier (1964) at 25°, 17°, and 4°C and fed with *Tetrahymena pyriformis*. In most of the experiments the amoebae were kept for 24 h in the presence of food, and for 24 h in a fresh culture medium without food. In one case only, the amoebae were maintained for 48 h with food, and for 48 h without food. In all the cases the culture medium was changed daily.

The nuclear DNA content was measured in synchronized amoebae. The absence of correlation between the DNA contents in the nuclei of sister cells 1 h after division at 17° ($r = 0.099$, $n = 16$) enabled us to use synchronized samples disregarding the amoeba origin. To obtain such samples, dividing cells (the so-called "division spheres") were selected with a pipette from a mass culture. Daughter cells picked up in 30 min were placed separately into microaquaria and given food. The nuclei were isolated by pipetting the cells in a solution with Triton X-100 and spermidine (Prescott et al. 1966) diluted four times with amoeba culture medium. The isolated nuclei were transferred with a micropipette in a drop of fluid onto a clean slide preliminarily ruled in squares with a corundum marker.

The amoebae lack a G_1 phase and the earliest possible time for isolation of nuclei is 1–1.5 h after division, when the reconstruction of daughter nucleus envelope is completed (Roth et al. 1960). Therefore, it is impossible to determine accurately the true postmitotic content of nuclear DNA for these protozoans. Nuclei isolated 1–1.5 h after division are here arbitrarily called postmitotic. Every 5 nuclei were air-dried inside a square and fixed with methanol. After the isolation of all nuclei belonging to several experimental variants onto a single slide, they were additionally fixed with methanol for 1 min and air-dried. Each variant was usually repeated three times on different slides, together constituting a series. Nuclei on 3 slides of one series were stained simultaneously using a fluorescent variant of the Feulgen reaction (Rosanov and Kudryavtsev

1967, Kudryavtsev and Rosanov 1974). Auramine-SO_2 was used as a Schiff-type reagent (Kasten 1961).

The DNA content of individual nuclei was measured with a double-beam pulse cytofluorometer RIF-1 (Papayan et al. 1974, Kudryavtsev et al. 1979) under the same conditions as in Makhlin et al. (1979 a). The DNA content of the nuclei was shown by the arithmetic mean and its error. When calculating the average content of nuclear DNA for each variant of an experimental series, nuclei with atypical DNA content, deviating significantly from the bulk of the values, were excluded. The number of such nuclei in different variants of one series did not exceed 0.5% of all the nuclei isolated onto slides. A comparison was made only between the means characterizing the nuclear DNA content of different variants of one series, i.e., the nuclei isolated onto slides at different time but stained simultaneously. To estimate differences in the nuclear DNA content the 0.05 level of significance at Student's t-test was used.

Results

In *A. proteus* strain B the mean generation time (from one division to another) under the adopted feeding regime (24 h with and 24 h without food) at 17° and 25° is about 71 h and 43 h, respectively (Sopina 1978). Table 1 presents the DNA content in the nuclei of

Table 1

DNA content of nuclei of postmitotic and premitotic amoebae at different cultivation temperatures

Cultivation temperature (°C)	Time after division (hours)	No. of nuclei with DNA content measured	Mean DNA content per nucleus, arb. units ($\bar{x}\pm S_{\bar{x}}$)
17	1	98	107±4
	72	76	268±7
25	1	121	112±3
	43	90	247±2

postmitotic and premitotic amoebae cultured at 17° and 25°. Evidently, at these temperatures the nuclei contain equal DNA amounts 1 h after division, while the premitotic DNA content is different. At 17° the nuclear DNA content 72 h after division exceeds by 2.5 times that 1 h after division. At 25° premitotic nuclei (43 h after division) contain 2.2 times more DNA than the postmitotic nuclei (1 h after division).

In amoebae transferred from 17° to 4° immediately after the division, the nuclear DNA content increases gradually within the first two weeks of incubation at the low temperature, reaching a double level in a fortnight. It remains constant, however, during the next two

weeks (Table 2). Thus, unlike amoebae cultured constantly at 17° and 25,° amoebae transferred from 17° to 4° just after the division contain

Table 2

DNA content of nuclei of amoebae transferred from 17° to 4°C immediately after division

Time of maintenance at 4°C	No. of nuclei with DNA content measured	Mean DNA content per nucleus, arb. units ($\bar{x} \pm S_{\bar{x}}$)
1.5 h	90	114±1
48 h	27	143±2
72 h	64	176±2
1 week	92	199±2
2 weeks	86	214±3
4 weeks	90	212±2

nearly the doubled amount of nuclear DNA. It should be also stressed that the DNA content in nuclei 1.5 h after division is lower in amoebae transferred from 17° to 4° (114 ± 1 arb. units) than in amoebae grown at 17° (128 ± 1 arb. units). Since the DNA synthesis is probably much slower at 4° than at 17°, the DNA content of the nuclei of amoebae transferred immediately after division from 17° to 4° and fixed 1–1.5 h later is the nearest value to the true postmitotic content of nuclear DNA.

After the transfer of amoebae cultured during a fortnight at 4°, to 25°, the DNA synthesis is resumed in their nuclei. After a 24 h incubation at this temperature in the presence of food, the nuclear DNA content of such amoebae increases by 8%, and 24 h later, but without food, by 18% as compared with the initial doubled level. 48 hours after the transfer it is found to exceed 2.5 times the postmitotic (1 h after division) DNA content of amoebae cultured constantly at 25° (Table 3). Within 48 h at 25°, when food is introduced into the

Table 3

DNA content of nuclei of amoebae at 4°C and after their transfer from 4° to 25°C for 48 h (24 h with and 24 h without food)

Cultivation temperature (°C)	Time of maintenance at the given temperature	No. of nuclei with DNA content measured	Mean DNA content per nucleus, arb. units ($\bar{x} \pm S_{\bar{x}}$)
4	2 weeks	66	205±2
25	24 h	76	225±2
25	48 h	71	276±3

culture medium in 24 h, the DNA content of the nuclei of transferred amoebae increases totally by 26% and even exceeds the normal level occurring by the end of the interphase (43 h after division) in amoebae kept constantly at 25°. At the same time, it does not differ from the DNA content of premitotic nuclei of amoebae kept at 17°. In this case, during the first 24 h of incubation at 25°, the amoebae do not divide, and by the 48th hour division is resumed in 10% of the cells.

In another series of experiments, the nuclear DNA content in amoebae transferred to 25° after a two-week incubation at 4° increased during 48 h, depending on the adopted feeding regime, by 56–69% as compared with the initial nearly doubled level (Table 4). The DNA

Table 4

DNA content of nuclei of amoebae at 4°C and after their transfer from 4° to 25°C for 48 h with different feeding regimes

Cultivation temperature (°C)	Feeding regime	Time of maintenance at the given temperature	No. of nuclei with DNA content measured	Mean DNA content per nucleus, arb. units ($\bar{x} \pm S_{\bar{x}}$)
25	48 h with food	24 h	120	202±3
		48 h	127	247±1
	48 h without food	24 h	122	188±2
		48 h	113	228±2
4	24 h with and 24 h without food	1.5 h	78	80±2
		2 weeks	95	146±2

content of the nuclei of amoebae maintained continuously in the presence of food exceeded 3.1 times the postmitotic (1.5 h after division) nuclear DNA content of amoebae kept at 4°, while that of starving amoebae exceeded the same value 2.8 times. Both in 24 h and in 48 h the DNA content of the nuclei of starving amoebae was lower than that of the nuclei of amoebae constantly supplied with food. Starving amoebae do not divide within 48 h at 25°.

After the transfer of premitotic amoebae (72 h after division) from 17° to 4°, the DNA content of their nuclei remains constant over a week, and in two weeks it is somewhat lower than that at the end of the interphase in amoebae cultured constantly at 17° (Table 5). Despite the fact that the nuclear DNA content of premitotic amoebae transferred from 17° to 4° for two weeks is already increased more than two times, these amoebae do not divide at least during a fortnight.

Table 5

DNA content of nuclei of premitotic amoebae at 17°C and after their transfer from 17° to 4°C for two weeks

Cultivation temperature (°C)	Time of maintenance at the given temperature	No. of nuclei with DNA content measured	Mean DNA content per nucleus, arb. units ($\bar{x} \pm S_{\bar{x}}$)
17	72 h	76	268±7
4	1 week	72	265±6
4	2 weeks	83	250±5

Discussion

The above data show that 1 h after division, the amoebae cultured at 17° and 25° have the same nuclear DNA content whereas it is different in premitotic nuclei. At 25° the amount of nuclear DNA increases 2.2 times during the interphase and at 17°, 2.5 times. This fact suggests that a certain excess amount of nuclear DNA is synthesized at both temperatures during the cell cycle, as compared with the value expected after a single replication. Similar results were obtained earlier for strain C of *A. proteus* cultured at 25°. It has been supposed that during a cell cycle the nuclear DNA content of the amoebae increases more than twice due to the amplification of genes coding for rRNA (Makhlin et al. 1979 a). As it was done earlier (Makhlin et al. 1979 a), we shall call DNA which is replicated more than once, extra DNA.

Cultivation of *Paramecium caudatum* at 4–5° is known to raise the DNA content of their macronuclei (Kovaljeva 1963, Kovaljeva and Selivanova 1963, 1966). Prolonged incubation of the flagellate *Euglena gracilis* at 4° is also accompanied by a considerable increase of the nuclear DNA content as compared with euglenas cultured at 22–25° (Sukhanova et al. 1979). Unfortunately, all these data have been obtained for samples of non-synchronized protozoan cells. In amoebae, the acclimation to 4° involves quite a different change in the nuclear DNA content as compared with the ciliate and flagellate species studied. If amoebae cultured at 17° are transferred to 4° immediately after the division, their nuclear DNA content is only doubled during a fortnight. Then, for at least the next two weeks, this DNA content remains constant. After the transfer from 17° to 4° of premitotic amoebae, the amount of DNA in which is already more than doub-

led as compared with the postmitotic level, the DNA content of their nuclei does not change during a week of incubation at the low temperature. After the next week it is found to be somewhat lower than the initial level. However, it does not differ significantly from the DNA content in amoebae 1 week after their transfer to 4°. As shown by electron microscopic autoradiography, a preferential ^{3}H-thymidine incorporation during the second half of the *A. proteus* cell cycle occurs into the peripheral zone of the nucleus, where numerous nucleoli are localized (Minassian and Bell 1976). Therefore, it is not excluded that only a single replication of chromosomal DNA takes place at 4°, due to resources accumulated by the cell during the previous cycle, but no synthesis of nucleolar DNA proceeds. Obviously, to demonstrate that nucleolar DNA is not amplified at 4° it is necessary to use autoradiography techniques.

After the transfer of amoebae cultured at 4° during a fortnight to 25° the initial doubled amount of DNA in their nuclei increases further within 48 h (24 h with and 24 h without food), reaching a level which even exceeds the value characteristic of the end of interphase in amoebae constantly maintained at 25°. It does not differ, however, from the premitotic content of nuclear DNA in amoebae cultured at 17°. The amount of extra DNA to be synthesized depends on the quantity of food consumed: 24 h and 48 h after the transfer from 4° to 25° it is lower in starved amoebae than in constantly fed ones. The data obtained allow the suggestion that during the amoeba cell cycle the synthesis of nucleolar DNA takes place after the completion of chromosemal DNA replication, i.e., these events do not coincide in time.

In the second half of the cell cycle, DNA synthesis starts also in cases when starved amoebae are refed (Prescott 1973, 1976 a, b, Makhlin et al. 1979 b, Spear and Prescott 1980). The DNA newly synthesized after refeeding of starved amoebae with ^{3}H-thymidine-labelled tetrahymenas was found to be mostly satellite. It was supposed that all or part of this satellite DNA (24% of the whole nuclear DNA) was rDNA (Spear and Prescott 1980). As shown cytofluorometrically, after refeeding of starving amoebae their nuclear DNA content increases up to the level recorded at the end of interphase in amoebae constantly supplied with food (Makhlin et al. 1979 b). This fact suggests that in amoebae the amplification of nucleolar DNA depends on food consumption after the division: starvation stops the rDNA replication, and refeeding restores its synthesis (Makhlin et al. 1979 b, Spear and Prescott 1980). Since the quantity of food introduced into the culture medium and consumed by the amoebae is not precisely assessed, differences in the synthesis of nucleolar DNA

might have been the cause of instability in the premitotic content of amoeba nuclear DNA.

It was not yet clear whether the absence of extra DNA synthesis in amoebae cultured at 4° is due to an immediate effect of low temperature or to starvation of the amoebae at this temperature. At 4° amoebae of strain B do not lose their ability to feed but the rate of phagocytosis is exceedingly low (Kovan'ko and Sopina 1977). After the transfer of amoebae maintained at 4° during a fortnight to 25° the DNA synthesis is resumed not only in feeding but also in starving amoebae and within 48 h their nuclear DNA content increases by 56–69% as compared with the initial nearly doubled level and thus exceeds 2.8–3.1 times the postmitotic DNA content at 4°. Extra DNA synthesis was observed also in starved amoebae maintained constantly at 25° (Makhlin 1981). Therefore, it may be taken for granted that the absence of extra DNA synthesis in amoebae cultured at 4° is due to the direct effect of low temperature and not to their starvation.

The results obtained support Prescott's idea (Prescott 1973, 1976 a, b) that the cessation of amoeba reproduction is caused by their arrest in the second half of the cell cycle. At 4° there is no synthesis of extra DNA and amoebae do not divide. 48 h after the transfer of amoebae with a doubled amount of nuclear DNA from 4° to 25° the DNA synthesis is resumed and division is restored in at least 10% of the cells. Starving amoebae do not divide under such conditions. After the transfer of amoebae from 4° to a temperature normal for their reproduction, the resumption of division seems to be correlated with a more than twice increase of nuclear DNA content up to a certain critical value. However, premitotic amoebae transferred from 17° to 4° do not divide at least during a fortnight, although their nuclear DNA content is already increased more than twice as compared with the postmitotic level. Consequently, the absence of extra DNA replication is not the only possible reason for blocking amoeba division at 4°. Decrease of ribosomes attached to membranes of the endoplasmic reticulum, increase of free monosomes, and also the presence in the cytoplasm of large groups of RNA-protein helices originating from the nucleus, seem to be signs of inhibition of protein synthesis in the amoebae at low temperature (Smith 1979). This may be also one of the reasons why amoeba division is blocked at 4°.

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Effects of Dinitrophenol, Actinomycin-D and Puromycin on Encystment of the Ameboflagellate, *Naegleria gruberi* *

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Synopsis. Populations of encysting amebas, *Naegleria gruberi*, were exposed to either 1×10^{-4}M dinitrophenol, or to 1×10^{-5}M actinomycin-d, or to 5×10^{-4}M puromycin in conditions that would otherwise (in controls) produce maximal encystment in 48 h. Dinitrophenol retarded the rate of encystment, but did not halt it, so that 67.3% of the population encysted in 48 h while in accompanying control populations over 80% had encysted. Puromycin completely blocked the encystment and no amebas progressed beyond the rounded, initial, precystic state and no cyst walls were formed. Actinomycin-d accelerated the rate of encystment so that 95.25% of the amebas had encysted within 10 h whereas in accompanying control populations only 28% had encysted within 10 h and 72.5% had encysted in 48 h. It is suggested that dinitrophenol reduces the rate of encystment by reducing the amount of available adenosine triphosphate (ATP) and thereby reduces the amount of available energy to support the enzyme-systems involved in the encystment processes. Puromycin is considered to inhibit transcription of the messenger-RNA codes, thereby preventing formation of the enzyme-systems required to metabolize and polymerize the cellular materials into the structures of the cyst walls. Actinomycin-d is considered to stimulate the release of stored messenger-RNAs from the nucleolus, thereby accelerating the transcription of and polymerization of the enzyme-systems required to promote the formation of the cyst. Cyclic adenosine monophosphate (cAMP) is suggested as a possible intracellular messenger in these activities.

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The mechanisms of encystment of protozoa are little understood, as are also the specific conditions that trigger encystment (Corliss and Esser 1974). Some studies show that encystment is initiated by contact between cells of the species as they begin to starve, for example, the ameboflagellate, *Naegleria gruberi* (Chiovetti and Bovee 1982), but the nature of the signal is unknown, although it is suggested to be generated by the interaction of proteinaceous end-chains at the cell-surfaces upon contact (Chiovetti and Bovee l. c.) Cyclic adenosine triphosphate (cAMP) may be active in relaying the signal, since internal cAMP increases as encystment begins in the ameba *Acanthamoeba* ("*Hartmannella*") *culbertsoni* and externally applied cAMP stimulates its encystment, while either cyclohexamide or actinomycin-d administered externally with the cAMP blocks encystment (Raizada and Krishna-Murti 1972). The nature of the contact signal is thereby indicated to be proteinaceous, since both cyclohexamide and actinomycin-d inhibit protein synthesis. Further evidence for the proteinaceous nature of the contact sites that trigger encystment is that temporary exposure to trypsin temporarily inhibits the initiation of encystment (Chiovetti and Bovee l. c.).

In trying to elucidate this problem further, we have exposed the encysting amebas of the ameboflagellate, *Naegleria gruberi*, to concentrations of actinomycin-d, which inhibits the synthesis of proteins, and separately to puromycin, which inhibits the synthesis of ribonucleic acids (RNA) on which the synthesis of proteins depends, or to dinitrophenol, which inhibits oxidative phosphorylations and thus the use of adenosine triphosphate (ATP).

Materials and Methods

The amebas were grown and harvested as previously described (Chiovetti and Bovee l. c.). Amebas were plated for encystment in PS-TRIS [3] at concentrations of 2000/mm^2 on agar surface.

In experiments, the amebas, similarly concentrated, were exposed, in PS-TRIS, to either added 1×10^{-5}M actinomycin-d, or 5×10^{-4}M puromycin, or to 1×10^{-4}M dinitrophenol during 48 h. Each experiment was run in triplicate [4], the data were pooled and plotted for the three runs.

[3] Tris (hydroxymethylaminomethane hydrochloride) at pH 7.4

[4] The inoculum for each run of each experiment was calculated as derived from the surface area of the petri plate (2.21×10^3 mm^2) requiring a total of 2.21×10^6 organisms for a final density of 1000 trophozoites/mm^2. Each inoculum was calculated as follows:

$$\frac{2.21 \times 10^6 \text{ trophs}}{\text{density, trophs/ml of suspension}} = \text{volume (ml) of suspension to give } 2.21 \times 10^6 \text{ trophs on a 60 mm plate}$$

Either Chi-square contingency tables (Remington and Schork 1970) or R × C tests of association (Sokal and Rholf 1969) were used to assess the effects of experimentation. Mean values and standard errors of the means of replicates were computed on a Hewlett-Packard No. 35 calculator.

Observations and Results

Controls for the Dinitrophenol Experiments

On control plates, (Pl. I 1 a–c) 80% of the amebas encysted within 48 h, beginning with a lag period of 4–5 h, then 36% encysted within 9 h, (S.E. = 20.23), 62% had encysted within 19 h, (S.E. = 19.97), 67% had encysted within 25 h, (S.E. = 15.71), 65% had encysted within 32 h, (S.E. = 6.24), and over 80% had encysted within 48 h (S.E. = 7.94). (Fig. 1).

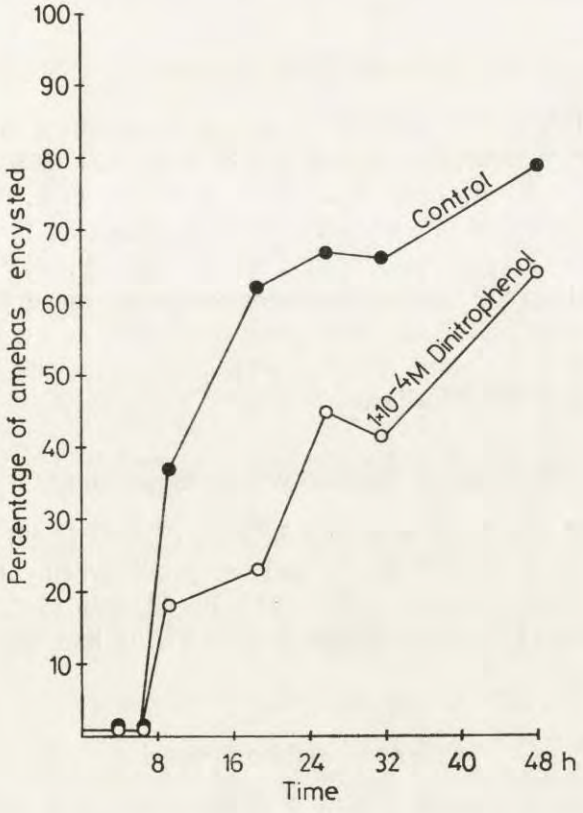

Fig. 1. Delay of encystment of the amebas caused by exposure to dinitrophenol compared to the control group

Effects of Dinitrophenol

Dinitrophenol significantly delayed encystment. At 7 h, 18% were precystic (S.E. = 6.56); within 19 h, nearly 24% had encysted (S.E. = 6.11); within 25 h, 44% had encysted (S.E. = 15.13), within 31 h, 40.3% had encysted (S.E. = 8.33), and within 48 h, 67.3% had encysted (S.E. = 8.33). (Fig. 1). The cysts were normal in appearance and structure.

Controls for Puromycin Experiments

Less than 2% (S.E = 2.45) had encysted within 8 h, 44.6% (S.E. = 12.10) had encysted within 12 h, 54.25% (S.E. = 11.44) had encysted within 20 h, 57.75% (S.E. = 13.15) had encysted within 30 h, 70.5% (S.E. = 8.89) had encysted within 34 h, and 81% had encysted (S.E. = 11.63) within 46 h.

Effects of Puromycin

Less than 1% (S.E. = 0.82 were precystic within 8 h, only 7.75% (S.E. = 4.35) were precystic within 12 h, only 4% (S.E. = 0.81) were precystic within 20 h, only 5% were precystic (S.E. = 3.36) within 30 h, only 4% (S.E. = 0.82) were precystic within 34 h, and only 2% (S.E. = 0.82) were precystic within 46 h. (Fig. 2). Cysts never fully developed! The cells never produced more than the thin, proteinaceous ectocyst of the late precystic state, as evidenced by interference light microscopy (Pl. I 2 a–c). Those precysts lysed quickly (within 3.5 min) in TRIS buffer.

Controls for Actinomycin-d Experiments

No cysts had developed within 5 h, 28% (S.E. = 18.49) had encysted within 10 h, 46% (S.E. = 15.43) had encysted within 15 h, 58.75% (S.E. = 14.38) had encysted within 26.5 h, 66.35% (S.E. = 12.82) had encysted within 35 h, and 72.5% (S.E. = 13.79) had encysted within 48 h. (Fig. 3).

Effects of Actinomycin-d

No cysts had developed within 5 h, but 94% (S.E. = 2.83) had encysted within 10 h, 95.25% (S.E. = 1.71) had encysted within 15 h, 97.25% (S.E. = 1.71) had encysted within 20 h, 98.5% (S.E. = 1.29) had

encysted within 26.5 h, 98.5% (S.E. = 1.29) had encysted within 35 h, and 99% (S.E. = 0.82) had encysted within 48 h. (Fig. 3). These cysts were normally viable, with excystment also normal. The cysts usually had only a thin, proteinaceous ectocyst, but had a denser cellulose endocyst than did the controls, as seen in both interference light microscopy (Pl. I 3 a, b).

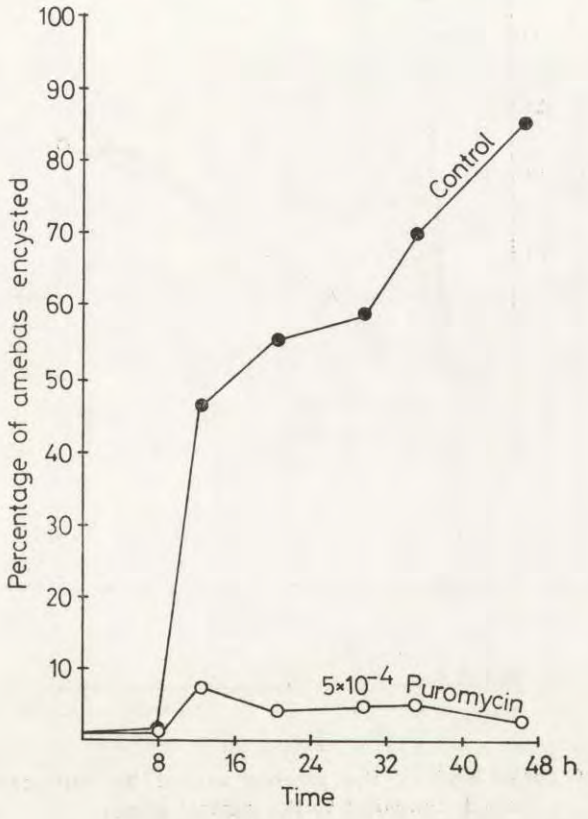

Fig. 2. Delay of encystment of the amebas caused by exposure to puromycin compared to the control group

Discussion

Metabolic inhibitors are widely used to interrupt various cellular processes and thereby help to elucidate the sequences of cellular events. Dinitrophenol is known to uncouple the diphosphoesterases in the conversion of ADP to ATP preventing the formation of ATP through the

mitochondrial cytochrome system (McGilvery 1970), thereby limiting the amount of available ATP and energy therefrom for metabolism. Alternate pathways apparently are still functional since dinitrophenol slows, but does not stop encystment.

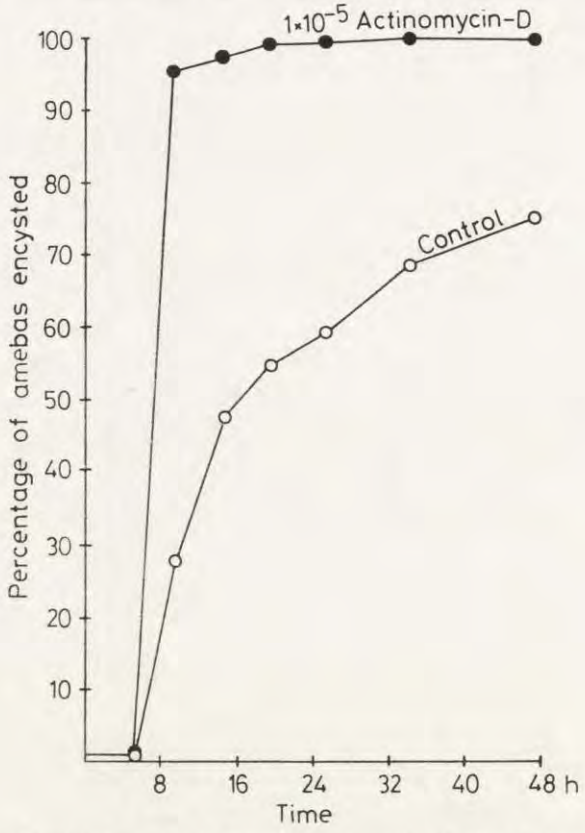

Fig. 3. Increase of encystment of the amebas caused by exposure to actinomycin-d compared to the control group

The results of the use of dinitrophenol in these experiments suggest two things: (1) that induction of encystment is not an energy-requiring step, since encystment begins at about the same time (within 8 h) for both the controls and experimentals (Fig. 1), and (2) the slowed rate of encystment during 48 h compared to controls, clearly shows that the rate of development of the cyst is dependent on the available energy-supply (as ATP), since the number of cysts that can fully develop in that period of time is much less than the number that develop in the control-population. Since Raizada and Krishna-Murti (1972) have shown that the synthetases involved in cyst-formation are

anaerobically active, the amount of available ATP should, as our results show, regulate the rate of encystment, and any reduction in the supply of ATP (as here affected by dinitrophenol) should (and does) reduce the rate of encystment.

Our experiments with puromycin, which restricts the transcription of the messenger-RNA code by blocking peptidyl-transferases (McGilvery 1970) suggest that the enzyme-systems (synthetases) involved are produced after encystment has been triggered and are dependent for their presence upon the action of newly-produced enzymes and not on a pre-existing, cytoplasmic pool of enzymes. Puromycin almost completely restricts the formation of all but a very few cysts. Neither protein synthetases nor carbohydrate synthetases appear to be produced, since the cells never progress beyond a fragile, precystic state. Internally, no autophagic vesicles (always present in normally precystic amebas) develop in those treated with puromycin. This indicates that (1) no hydrolases are produced, that (2) materials needed for cyst-wall production are not produced, nor polymerized, due to the failure of the puromycin-treated cell to produce the needed enzymes.

Our experiments with actinomycin-d, which inhibits protein synthesis by blocking the messenger-RNA producing action of DNA, prevented the complete formation of the thin, protein ectocyst, but greatly accelerated the rate at which the thick, cellulose endocyst developed. These results suggest that pools of mRNAs exist, perhaps in the nucleolus (since that decreases in size as encystment proceeds) and that those are rapidly released into the cytoplasm and used in the production of the hydrolases and synthetases required to convert protoplasmic metabolites into the polymers that are built into the cyst walls.

Some activating substance may be involved, but we do not know what it is. One possibility is cyclic adenosine monophosphate (cAMP), since that has been shown to stimulate encystment of *Acanthamoeba* ("*hartmannella*") *culbertsoni* (Raizada and Krishna-Murti 1972) and the aggregation, migration and encystment of the acrasid amebulae of the "slime-mold", *Dictyostelium discoideum* (Konijn et al. 1968).

Many steps in the encystment-process of *Naegleria gruberi* remain to be chemically elucidated.

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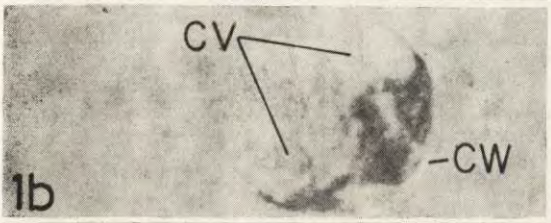
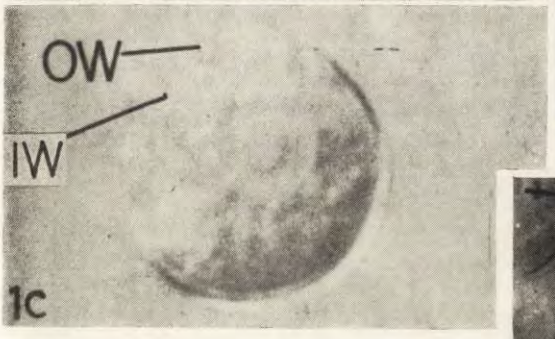
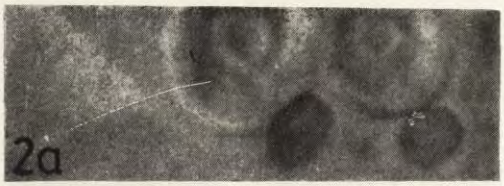
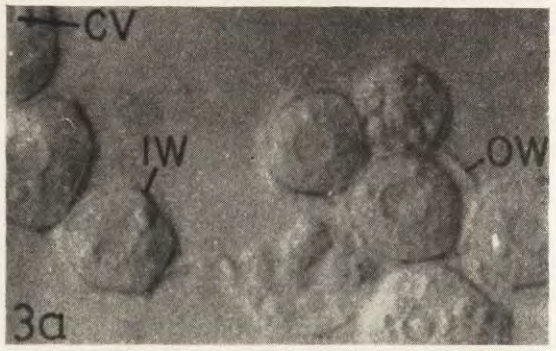
EXPLANATION OF PLATE

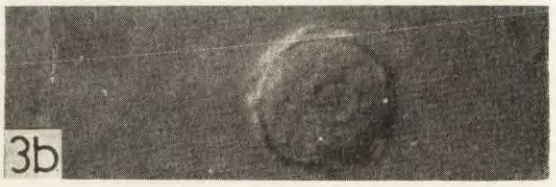
1: Normally developed cysts of *Naegleria gruberi*
1 a: Rounded, early, pre-cystic stage of *Naegleria gruberi*, no cyst walls are present
1 b: Early encystment. The outer cyst-wall (CW) has formed. Cytoplasmic vesicles (CV) are numerous
1 c: A mature cyst. The cyst is round, smooth, with a thin outer wall tightly against the thicker inner wall (IW). Fewer cytoplasmic vesicles are present
2: Failure of *Naegleria gruberi* to form cysts after exposure to puromycin
2 a: Rounded pre-cystic stage, no cyst-walls are formed
2 b: The same cells after exposure to TRIS for 3 min. Some cytoplasm has leached out of the cells (CD — cytoplasmic debris)
2 c: After 3.5 min exposure to TRIS. More cytoplasm has erupted from the cells as well as a nuclear fragment (NF)
3: Cysts formed after exposure of *Naegleria* to actinomycin-d
3 a: A group of cysts, the outer wall of the cysts (OW) are fragmented and torn or are separated from the inner walls (IW) Most cysts still contain cytoplasmic vesicles (CV). These cysts are viable
3 b: A single cyst, outer wall is fragmented, inner wall is intact, nucleus seen in center of cyst, few cytoplasmic vesicles











R. Chiovetti, Jr. et E. C. Bovee auctores phot.

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Effect of Various Levels of External pCa on the Barium-Induced Motor Responses of *Paramecium caudatum*

Received on 7 July 1982

Synopsis. The influence of the absolute Ca^{2+} concentration in the medium on the barium-induced motor responses of *P. caudatum* was investigated. Motor reactions of paramecia were recorded by means of long-exposure dark-field photomacrographic technique. Series of experiments were performed at levels of external calcium 1 mmol $CaCl_2$ (pCa 3) and 10 mmol $CaCl_2$ (pCa 2). When the ratio $[Ba_0^{2+}]$: : $[Ca_0^{2+}]$ was 0.25 both at pCa 3 and pCa 2, more than 80% of ciliates showed Periodic Ciliary Reversal (PCR), while in the range of ratio 0.25–2 at pCa 3 the percentage of paramecia responding with PCR decreased and that of showing Continuous Ciliary Reversal (CCR) increased. However, at pCa 2 the percentage of ciliates showing PCR was more or less constant in the whole range of $[Ba_0^{2+}]$: $[Ca_0^{2+}]$ concentration ratio with slight increase of number of animals responding with CCR at higher values of ratio. The obtained results support the hypothesis of inactivation of the membrane calcium channels by calcium deposited at the inner side of ciliary membrane.

The response of *P. caudatum* towards cation stimulation is manifested at the beginning by backward swimming, i.e., ciliary reversal. Both the appearance of this reaction and its duration depend on the acting cation : calcium concentration ratio in the medium. When this ratio is relatively low, the backward swimming lasts shorter time and changes frequently into forward swimming (PCR — Dryl (1961)). The increase of the ratio prolongs the backward movement (CCR — Dryl (1961), Kuźnicki (1963)).

The mechanism of excitation depends on the influx of Ba^{2+} and Ca^{2+} into the cell (Brehm and Eckert 1978, Ling and Kung 1980). Increase of Ca_i^{2+} over 10^{-6} mol evokes ciliary reversal (Naitoh and Kaneko 1972). Both ions are believed to enter the cell through

the calcium channels located in the ciliary membrane (Dunlap 1977). The functional state of the calcium channels seems to be an important factor controlling the excitation. Ionic competition on the outer side of the membrane is believed to be a regulatory mechanism of closing and opening of the calcium channels (Naitoh 1968, Hildebrand 1975).

Hildebrand and Dryl (1976) postulate also the existence of an ionic exchange system which controls the membrane conductance and is located on the inner side of the membrane. According to these authors elevation of internal calcium concentration should inactivate the calcium channels from inside. Some electrophysiological experiments of Brehm and Eckert (1978) are in agreement with that concept. The aim of the present paper was to check the validity of the above mentioned hypothesis.

Material and Methods

Paramecium caudatum, grown in the meadow hay culture, before the experiments was washed three times in 1 mmol Tris/HCl, 1 mmol $CaCl_2$ solution (pH 7.2). After washing the animals were adapted to pH 7.0 and desired pCa in the maleic acid buffer containing $CaCl_2$ (5 mmol Tris/maleic acid + 5 mmol NaOH + 1 mmol $CaCl_2$ or 10 mmol). As a stimulation agent $BaCl_2$ was used. It was solved in the buffer solution (0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2 mmol in pCa 3 series of experiments and 2.5, 5, 7.5, 10, 12.5, 15, 17.5, 20 mmol in pCa 2 one).

The movement of paramecia was recorded using long-exposure dark-field photomacrographic method (Dryl 1958). The recording started 4 min after application of $BaCl_2$ and lasted 5 s. Ten repetitions for every $BaCl_2$ concentration were performed. Mean and standard error were calculated (at confidence level 95%). The experiments were carried out at 21 ± 1°C.

Results

In the solution containing no $BaCl_2$ the percentage of paramecia swimming forward (FLS — Forward Left Spiraling movement, Dryl and Grębecki (1965)) is high (68–87%). Introduction of $BaCl_2$ to the solution in the concentration ratio to $CaCl_2$ from 0.25 to 2 decreases the percentage of animals showing FLS from 17% to 0%. There are only slight differences in the percentage of FLS between pCa 2 and pCa 3 (Fig. 1).

Under Ba^{2+} stimulation the majority of *P. caudatum* showed both kinds of backward swimming: PCR (Fig. 2) and CCR (Fig. 3). The per-

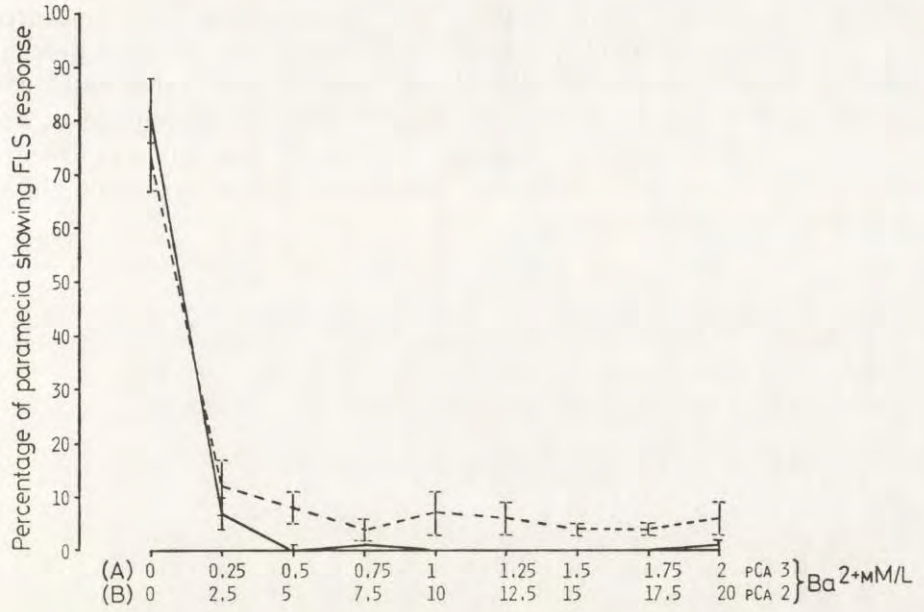

Fig. 1. Percentage of *P. caudatum* showing FLS in the solution containing different Ba^{2+} concentrations: a — in pCa 3 — solid line, b — in pCa 2 —dashed line. The standard error is marked. A — Concentrations of $BaCl_2$ at pCa 3, B — Concentrations of $BaCl_2$ at pCa 2

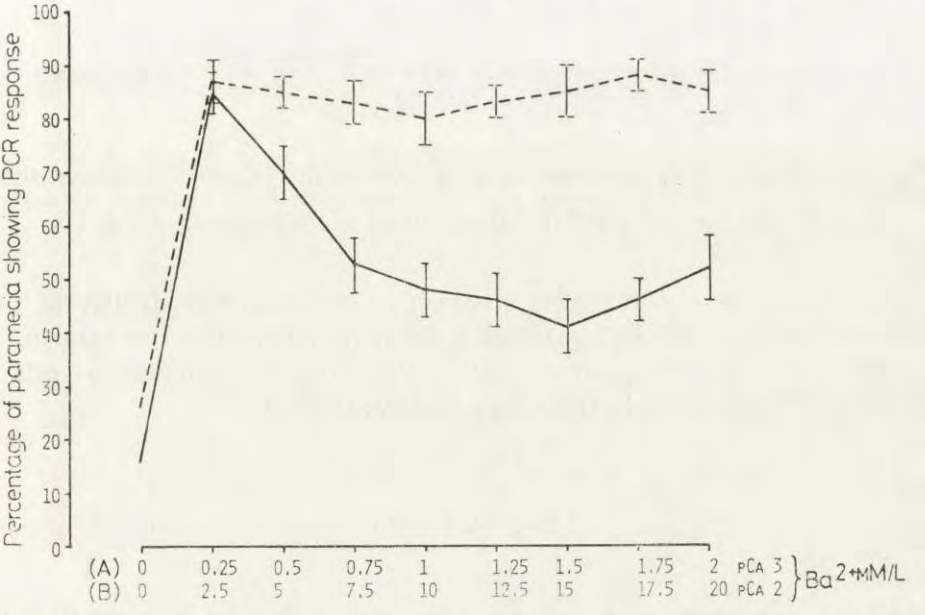

Fig. 2. Percentage of *P. caudatum* showing PCR in the solution containing different Ba^{2+} concentrations: a — in pCa 3 — solid line, b — in pCa 2 — dashed line. The standard error is marked. Other explanations as in Fig. 1

centage of paramecia showing PCR was maximal when the concentration ratio $[Ba_0^{2+}] : [Ca_0^{2+}]$ was 0.25. The value of the maximal percentage was the same independently of the absolute Ca_0^{2+} and Ba_0^{2+} concentrations. For pCa 2 in the ratio range 0.25–2 the percentage of animals showing PCR was kept almost constant, for pCa 3 it was decreased in the ratio range 0.25–0.75 and the minimal level was kept almost constant up to the ratio 2 (Fig. 2).

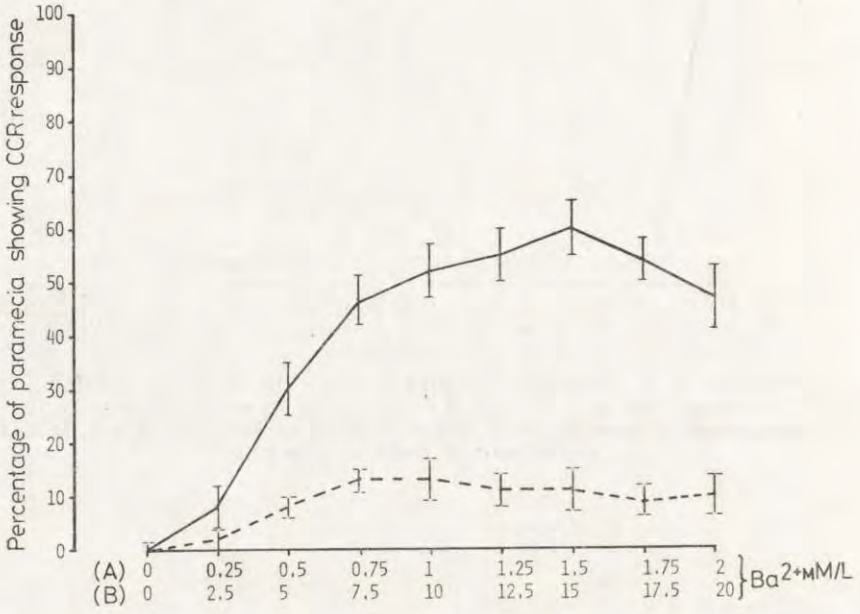

Fig. 3. Percentage of *P. caudatum* showing CCR in the solution containing different Ba^{2+} concentrations: a — in pCa 3 — solid line, b — in pCa 2 — dashed line. The standard error is marked. Other explanations as in Fig. 1

The percentage of animals showing CCR increased parallel to the increase of the ratio and reached a constant level over the ratio 0.75. The maximal percentage of animals showing CCR was higher at pCa 3 and the difference was in the range 38–55% (Fig. 3)

Discussion

Slight difference in the percentage of individuals showing FLS in dependence on pCa might be caused by different levels (although not statistically significant) of spontaneous motor reactions in the control

conditions in medium devoid of Ba_0^{2+} (Fig. 1). The percentage of paramecia swimming backward spontaneously in the absence of Ba^{2+} could be affected by pCa or/and could be the result of other factors which may undergo uncontrolled change during the experiment.

The percentage of PCR and CCR at given $[Ba_0^{2+}] : [Ca_0^{2+}]$ ratio was different depending on pCa. These differences might be explained by blocking the calcium channel on the inner side of the membrane as postulated by Hildebrand and Dryl (1976). The tenfold increase of Ca_0^{2+} concentration in pCa 2 experiments was correlated with tenfold increase of Ba_0^{2+} concentration, so that concentration ratio $[Ba_0^{2+}] : [Ca_0^{2+}]$ was kept constant. It means that competition relations between Ba^{2+} and Ca^{2+} outside the cell as well as the number of opened channels should also be unchanged. But the increase of the absolute concentrations of Ba_0^{2+} and Ca_0^{2+} leads probably to augmentation of the influx of these ions through the single channel. So in pCa 2 environment because of increased influx the accumulation rate of Ca_i^{2+} is higher comparing with pCa 3 conditions. It is possible that accumulation rate of Ca_i^{2+} could influence the level of Ca_i^{2+} concentration at which the calcium channels are blocked from inside. When the accumulation is rapid the critical Ca_i^{2+} concentration is relatively low. Due to the presented theory the longer lasting backward swimming at certain ratio $[Ba_0^{2+}] [Ca_0^{2+}]$ at pCa 3 could be the result of stronger Ca^{2+} accumulation in the cillium and longer exhursting time of it. Results presented in this paper are in agreement with experiments of Ling and Kung (1980). Using radioactive $^{133}Ba^{2+}$ they have measured directly the influx of barium ions to the cell of *P. tetraurelia*. By increasing Ba_0^{2+} and Ca_0^{2+} concentrations (keeping the ratio $[Ba_0^{2+}] : [Ca_0^{2+}]$ constant) the influx of Ba^{2+} was decreased. The dependence of Ba^{2+} influx on the absolute Ba_0^{2+} and Ca_0^{2+} concentrations was observed only when the concentration ratio of these ions did not exceed 5.5. When it was over 5.5 the influx depended only on the ratio value. In view of the presented above hypothesis when the ratio is over 5.5, probably the number of Ca^{2+} entering the cell with Ba^{2+} is not high enough to inactivate the calcium channel.

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Nouvelles observations sur une grégarine du genre *Selenidium* Giard, 1884, parasite de *Glossobalanus minutus* Kow. *(Entéropneusta, Hemichordata)*

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Synopsis. Dans ce travail est présentée une description en microscopie optique de différentes phases intra- et extraépitheliales du cycle d'une grégarine du genre *Selenidium* Giard, 1884, trouvée dans les diverticules hépatiques de *Glossobalanus minutus* Kow. De plus, on établit une relation entre cette grégarine et les deux espèces préalablement décrites chez des *Entéropneustes*, *S. metchnikowi* Léger et Duboscq, 1917 et *S. grassei* Théodoridès et Desportes, 1968.

La présence de grégarines chez les Entéropneustes fut signalée par Spengel (1893) chez *Balanoglossus kuppferi* v. Will. Shum. Dans les deux cas, elles furent attribuées au genre *Monocystis.*

Plus tard, Léger et Duboscq (1917) découvrirent dans la région hépatique et génitale de *Glossobalanus minutus* Kow. une Archigrégarine qu'ils inclurèrent dans le genre *Selenidium* Giard, 1884, pour ses caractéristiques et à laquelle ils attribuèrent le statut de nouvelle espèce, *S. metchnikowi.*

Dans l'intestin de *Balanoglossus clavigerus*, Théodoridès et Desportes (1968) localisèrent des stades intra-et extracellulaires semblables à ceux décrits par Léger et Duboscq. En présentant cependant quelques différences avec ces derniers, ils créèrent une nouvelle espèce, *Selenidium grassei.*

D'autre part, les espèces appartenant au genre *Selenidium* sont fréquemment parasites des Annélides Polychètes (Caullery et Mesnil 1899, 1901, Reed 1933, Ray 1930, Mackinnon et Ray 1933, Tuzet et Ormiéres 1964, Levine 1971, Schrével 1971 a et b). Chez des Ascidies, Harant (1931) avait décrit une espèce,

Selenidium giganteum que, plus tard, Ormiéres (1964) détermina comme étant une coccidie.

Le présent travail étudie les différentes phases du cycle d'une Archigrégarine du genre *Selenidium* observée dans des coupes préparées pour l'étude de la région hépatique de *Glossobalanus minutus* (Fernández et Benito).

En plus de données sur la morphologie, la cytologie et le cycle de vie, quelques données histochimiques sont apportées.

Finalement on discute la valeur taxonomique des caractères précédents, vu que les exemplaires examinés pourraient être rapprochés de *Selenidium metchnikowi* ou bien de *Selenidium grassei.*

Matériel et méthodes

Les individus de *Glossobalanus minutus* Kow. utilisés dans cette étude, proviennent de la plage de Luanco (Asturias) et furent récoltés pendant les mois de décembre et de janvier.

Aprees trempage de quelques heures dans un récipient contenant de l'eau de mer afin d'éliminer le sable du tube digestif, ils sont fixés à basse température (0–4°) dans les solutions suivantes: Bouin, Gendre, Carnoy (6 : 3 : 1) et formol à 10% dans l'eau de mer.

Le matériel est inclu dans la paraffine et les blocs sont coupés en coupes de l'épaisseur de 5 à 8 µm.

Pour la coloration des coupes nous avons employé plusieurs procédés:

Colorations générales: Azocarmin de Heidenhain. Hematoxyline ferrique de Heidenhain. Trichromique de Mallory. Trichromique de Masson à la fuchsine acide et au bleu d'aniline. Picrofuchsine de Van Gieson.

Colorations histochimiques: Bleu Alcian (AB) pH 2.5 (Mowry). Bleu de Toluidine (TB) 0.01% pH 4.2. P.A.S. (Hotchkiss–McManus 1946). Bleu Alcian pH 2.5–P.A.S. (Mowry et Winkler 1956). Ninhydrine-Schiff (Yasuma et Itschikawa 1953). Tetrazoréaction de Danielli (1943), avec ou sans traitements préalables par le DNFB (dinitrofluorobenzene), oxydation performique, benzoïlation et désamination. Ferricyanure ferrique de Adams.

Résultats

Localisation du parasite:

Tous les stades observés, tant intra-qu'extraépithéliaux, ont été trouvés dans les diverticules de la zone postérieure de la région hépatique de *Glossobalanus minutus.*

Stades intraépithéliaux: Schizogonie

Les stades intraépithéliaux clairement schizogoniques, plus fréquents dans l'épithélium des diverticules hépatiques, correspondent à des formes sphériques de 5–8 μm de diamètre. Ils présentent un cytoplasme d'aspect homogène qui prend une coleur "fuchsia" d'intensité variable, avec le Trichromique de Masson et des tons rougues, jaunâtres ou bleuâtres avec l'azocarmin de Heidenhain. Le noyau, de 2–3 μm de diamètre, présente un nucléole très remarcable. (Fig. 1 b, c, Pl. I 3).

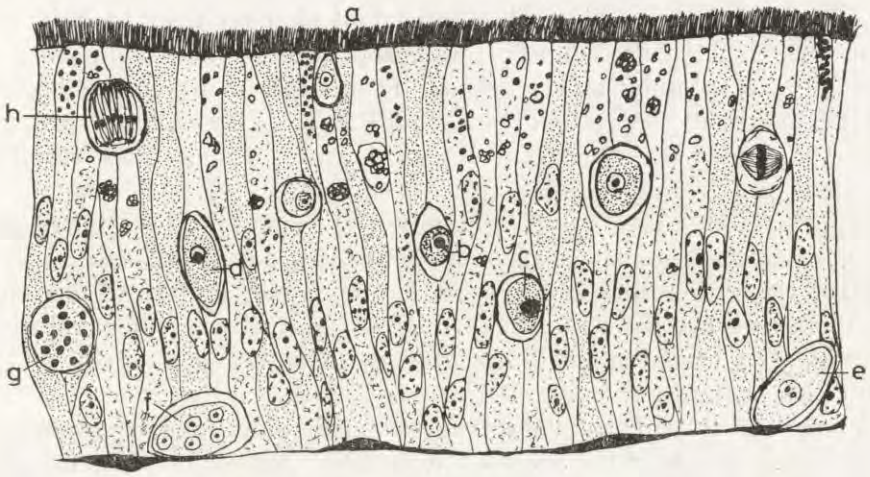

Fig. 1. Phases de la Schizogonie. (a, b, c, d, e — jeunes schizontes; f, g — stades plurinuclées; h — stade final ou "barrilet"

Ces structures s'observent à différents niveaux de l'épithélium, mais sont plus fréquents dans sa partie supérieure.

Dans quelques cas, nous avons observé des structures d'aspect piriforme dans la partie supérieure de l'épithélium, d'environ 7 × 3–4 μm, possédant un cytoplasme clair et homogène et un petit noyau. Nous pensons que ces formes représentent la première phase d'établissement du sporozoïte dans la cellule.

D'autre part, nous considérons les formes sphériques précédemment décrites comme les stades les plus jeunes provenant des sporozoïtes, ceux-ci modifiant leur forme immédiatement apres avoir pénétré dans la cellule.

En augmentant leur taille, les stades sphériques primitifs montrent souvent de différents aspects: oviformes, ovales et fusiformes. Ces structures plus dévelopées se rencontrent, en général, depuis la partie

centrale de l'épithélium jusqu'à sa base, bien qu'il y ait des exceptions, comme les structures oviformes de 9 × 6 µm qui sont situées dans le tiers supérieur de l'épithélium. Les formes allant jusqu'à 15 × 7 µm occupent le plus souvent la zone à proximité de la membrane basale et elles ont une forme plus ou moins ovale (Pl. I 4).

Dans tous ces stades de développement, le cytoplasme conserve son aspect uniforme et une coloration semblable aux phases sphériques primitives. Le noyau est plus grand, le nucléole atteignant jusqu'à 3 µm de diamètre.

Toutes ces structures uninuclées ont pu être observées dans les coupes avec des colorations histochimiques dont on peut tirer les faits suivants: avec le bleu de Toluidine, le cytoplasme se montre incolore ou teinté de bleu, plus ou moins intense; le nucléole étant très orthochromatique. Avec les colorations faites au P.A.S., le cytoplasme présente différents degrés de réaction, nuls ou infimes, jusqu'à des réactions nettement positives dans la plupart des cas.

Avec les techniques de détermination des aminoacides et des protéines, nous observons des réactions positives du cytoplasme avec le DNFB et la ninhydrine-Schiff. Dans les deux cas, l'intensité des colorations varie selon les structures. Ainsi on peut observer des réactions positives, bien que d'intensité variable, avec la tétrazoréaction de Danielli et les traitements préalables avec le DNFB.

Comme nous avons pu l'observer avec les colorations trichromiques et confirmer par la suite avec les techniques histochimiques, la différence d'affinité pour les colorants est une caractéristique commune à tous les stades uninuclées. Ceci met en évidence des modifications dans la nature du cytoplasme, probablement en relation avec son activité et les différentes phases de croissance.

Les stades uninucléés de plus grande taille qui se trouvent à la base de l'épithélium, entrent en multiplication par division nucléaire binaire. Ainsi, on observe des stades à 2, 6 et 12–14 noyaux (ce dernier stade présentant une forme presque sphérique et mesurant environ 11 × 9 µm).

La phase finale de la schizogonie montre des schizozoïtes disposés en "barrillet" que nous avons pu observer dans la partie supérieure de l'épithélium. Les schizozoïtes possèdent un noyau central et mesurent environ 8 µm de long. Ils sont au nombre de 12 à 14, bien que ce soit difficile de déterminer ce nombre avec exactitude. (Fig. 1 h, Pl. I 5).

Nous avons rencontré cette phase finale de la schizogonie uniquement dans les coupes colorées à la picrofuchsine de Van Gieson; la coloration noire de noyaux se détache sur le fond jaunâtre des schizozoïtes.

Stades extraépithéliaux: Gamogonie

Les stades extraépithéliaux les plus jeunes que nous avons observés, correspondent à des structures d'aspect oviforme ou légèrement piriforme, de 5 × 4–5 µm et de 6 × 4 µm. Ces formes correspondent à de jeunes gamontes qui se localisent soit dans la couche ciliée, soit légèrement fixés aux cellules de l'épithélium. Dans les premières phases de sa croissance, la jeune grégarine montre, en général, des formes ovales ou légèrement ovoides. Jusqu'à ce qu'elle atteigne une taille d'environ 12–14 µm × 10 µm, la plus grande partie de son corps est enfoncée dans l'épithélium. Peu à peu, en même temps que le gamonte croît vers la lumière des diverticules hépatiques, cette portion devient relativement plus petite. On peut observer des formes de 15 µm qui présentent la moitié du corps hors de la cellule. Pendant cette phase de croissance, le noyau, auparavant sphérique, devient ovale et occupe la quasi-totalité de l'axe transversal du gamonte. Le nucléole, trés remarquable, peut atteindre 3 µm de diamètre. (Fig. 2 A,B,C, Pl. II 6–8).

Le cytoplasme, dans les premiers stades de développement, présente un aspect finement granulé. Il prend des tons "fuchsia" avec le Trichromique de Masson et des tons bleus ou grisâtres avec l'azocarmin de Heidenhain. Au contraire, avec les préparations au bleu de toluidine, il se colore à peine ou prend des colorations bleues plus ou moins intenses.

En général, on obtient des réactions positives avec le P.A.S. et les colorations au ninhydrine-Schiff.

Le jeune gamonte grandit et croît jusqu'à la lumière du diverticule, restant ancré dans la cellule épithéliale par sa partie antérieure. Pendant cette phase de développement, la grégarine mesure 28 µm de longueur, dont 7 ou 8 restent dans la cellule. On trouve le noyau vers l'extrêmité postérieure du corps, occupant toute la largeur de la grégarine (jusqu'à 10 µm). (Fig. 2 D, Pl. II 9).

Avec un peu plus de 40 µm de long, la grégarine reste ancrée dans la cellule épithéliale et ses caractéristiques ne varient pas essentiellement par rapport au dernier stade décrit. (Pl. II 10). Son extrémité antérieure présente de petits corpuscules. (Fig. 2 E, F, Pl. II 11).

Dans une phase plus avancée du développement, le gamonte, mesurant 105 µm de long, reste légèrement attaché à l'épithélium par l'extrémité de sa partie antérieure, en forme de mucron. L'extrémité postérieure représente la zone la plus large de la grégarine, avec 13 µm. C'est dans cette dernière zone que l'on trouve le noyau avec sa forme ovale caractéristique. Dans ce stade de croissance, nous avons pu

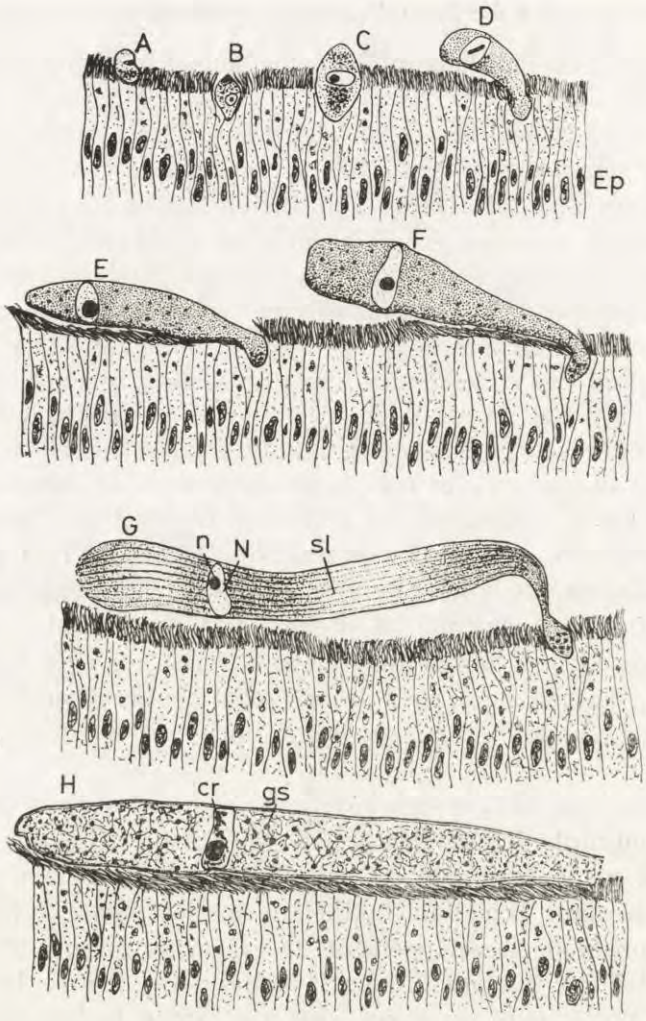

Fig. 2. Développement du gamonte. cr — granules de chromatine, sl — stries longitudinales, Ep — epithélium, gs — granules du cytoplasme, N — noyau, n — nucléole

voir les stries longitudinales, au nombre de 7 a 8 par face observée. (Fig. 2 G et Pl. II 11).

A 120–125 μm de long, le gamonte est libre dans la lumière du diverticule.

Dans des coupes colorées à l'azocarmin de Heidenhain et montrant une phase antérieure à celle-ci, le gamonte présentait un cytoplasme d'aspect réticulé et de petits granules colorés en rouge intense étaient

visibles entre les mailles; leurs tailles variant entre 0.5 et 1 µm.

Le noyau présente un nucléole de grande taille, environ 4 µm, sphérique, avec de petits granules chromatiques à sa périphérie. (Fig. 2 H, Pl. II 12, 13).

En ce qui concerne les colorations histochimiques, le nucléole aussi bien que les granules chromatiques se montrent très orthochromatiques avec le bleu de toluidine et ont une réaction positive à la tétrazoréaction de Danielli et aux procédés de tétrazo-benzoylation et de tétrazo-DNFB.

Dans la lumière des diverticules on peut observer fréquemment des coupes transversales et obliques de diverses grégarines, mais en aucun cas nous n'avons pu trouver de traces d'accouplement ou de syzigie.

Disscusion

Les différents stades intra- et extraépithéliaux observés dans les diverticules hépatiques de *Glossobalanus minutus* Kow. correspondent à un grégarinomorphe qui, par ses caractéristiques (cycle, morphologie, cytologie) est une Archigrégarine au sens de Grassé, 1959 du genre *Selenidium* Giard, 1884.

La position systématique des *Seleniidae* Brasil, 1907 est discutée par Schrevel (1971) qui donne une nouvelle définition de l'ordre des *Archigregarina* en se basant sur des observations biologiques et ultrastructurales et n'en y incluant que les grégarines parasites intestinales de Polychètes.

Levine (1971), crée une nouvelle famille, les *Selenidioidae*, dans l'ordre *Archigregarinorida*, transférant la famille des *Seleniidae* dans l'ordre des *Archigregarinorida*.

La famille des *Selenidioidae* contient le genre *Selenidioides* Levine, incluant les espèces qui, entre autres caractères, présentent un corps courbé ou en forme de demi-lune, sans cloison, foliacé ou cylindrique, avec des stries longitudinales, et qui possèdent des mérogonies et des gamontes à développement extracellulaire. Par ses caractères l'espèce *Selenidioides metchnikowi* (Levine), synonyme de *Selenidium metchnikowi* Léger et Duboscq, 1917, appartient à ce groupe.

Jusqu'à présent, chez les Entéropneustes, deux espèces appartenant au genre *Selenidium* Giard: *S. metchnikowi* Léger et Duboscq chez *Glossobalanus minutus* et *S. grassei* Théodoridès et Desportes, 1968, ont été décrites. Dans les deux cas les stades syzygie, gamétokyste et sporokyste n'ont pas été trouvés.

La description de ces espèces se base donc sur la schizogonie, la taille des trophozoïtes ou gamontes et le nombre des stries longitudinales du trophozoïte.

Théodoridès et Desportes tiennent également compte du type d'hôte, vu qu'il s'agit d'un genre différent de celui parasité par *Selenidium metchnikowi*, bien que ceux-ci appartiennent à la même famille des Entéropneustes.

L'observation des différents caractères, les uns très proches de ceux décrits pour *S. metchnikowi* et les autres semblables à ceux décrits pour *S. grassei*, nous a conduit à la réalisation de ce travail afin d'établir une relation avec les deux espèces. L'observation que nous avons faite chez *Glossobalanus minutus* apporte de nouvelles données sur le cycle de ce grégarinomorphe.

Nous avons toujours observé les différentes phases du cycle, tant intra-qu'extraépithéliales, dans les diverticules de la zone postérieure de la région hépatique (Fernández et Benito). Cependant, Léger et Duboscq localisèrent le parasite non seulement dans cette région, mais également dans la région génitale et mentionnèrent que la schizogonie fut toujours observée dans l'incision droite du sillon cilié. Théodoridès et Desportes, quant à eux, la localisèrent dans la région intestinale de *Balanoglossus clavigerus*.

Les stades intraépithéliaux que nous avons rencontrés dans les diverticules hépatiques rappellent ceux décrits par Léger et Duboscq, qui coincident en général avec nos observations. C'est ainsi le cas des stades ovoïdes de 10–11 μm de long qui entrent en multiplication et qui présentent plusieurs noyaux.

Cependant nous avons fréquemment observé des formes sphériques de 5 à 8 μm de diamètre à différents niveaux de l'épithélium qui n'ont pas été décrites précédemment.

Les formes ovoïdes et ovales se recontrent dans la moitié inférieure de l'épithélium, celles de plus grande taille occupant la partie la plus basale.

La phase finale de la schizogonie présente de 12 à 14 schizozoïtes, bien qu'il soit difficile d'en préciser le nombre exact. Léger et Duboscq donnent une marge plus grande, de 12 à 16 schizozoïtes, de 7 μm de long, c'est-à-dire, de taille semblable à ceux que nous avons observés ($\sim$ 8 μm).

Théodoridès et Desportes décrivent seulement l'état final de la schizogonie ou "barrilet" en y distinguant une douzaine de schizozoïtes de 12 μm de long.

L'existence de schizogonie dans ces parasites d'Entéropneustes, interprétée par Shrevel (1971) comme douteuse par rapport à l'es-

pèce décrite par Léger et Duboscq, est confirmée par Théodoridès et Desportes (1968) et corroborée par le présent travail.

En ce qui concerne les stades extracellulaires (trophozoïtes ou gamontes) nous avons observé des formes de très petite taille (6 × 4 μm) semblables à celles décrites chez *Selenidium metchnikowi*. Cependant, Léger et Duboscq regrettèrent de ne pas avoir rencontré des formes intermédiaires entre ces jeunes gamontes et ceux atteignant 30–34 μm de longueur.

Dans notre étude nous avons pu observer différentes phases de croissance des gamontes et établir une séquence de leur développement.

Léger et Duboscq comparèrent les jeunes gamontes de *Selenidium metchnikowi* à ceux de *S. caulleryi* décrits par Ray (1930) d'une taille de 6–8 μm × 4–6 μm, immobiles et intracellulaires.

Cependant, ces auteurs, bien qu'ils n'aient pu observer les stades intermédiaires, supposèrent que les gamontes de *S. metchnikowi* devaient être extracellulaires durant tout leur dévelopement.

En effet, les plus jeunes formes de la grégarine se situent, au début, sur la couche ciliée de l'épithélium. Ensuite elles pénètrent légèrement à l'intérieur de la cellule et continuent leur croissance jusqu'à la lumière du diverticule, retenues uniquement par l'extrémité antérieure de leur corps qui leur sert de point d'ancrage.

Théodoridès et Desportes prennent en compte, également, la taille des trophozoïtes pour considérer le *Selenidium* décrit chez *Balanoglossus clavigerus* comme une nouvelle espèce. Pour ceci, ils se basent sur le fait que *S. metchnikowi* est de taille inférieure à *S. grassei*. Ils considèrent les limites minimum et maximum, respectivement, de 5 a 30–40 μm pour la première espèce et de 60 à 120 μm de long, pour la seconde.

Cependant nous considérons ici que ce n'est pas un facteur dont on puisse tenir compte, car les formes que Léger et Duboscq décrivirent comme les plus grandes restent attachées à l'épithélium. Au plus, elles pourraient constituer des stades intermédiaires dans le développement de la grégarine.

D'autre part, la taille minimum que l'on a donné pour le trophozoïte de *Selenidium grassei*, correspond à des formes allongées reliées à l'épithélium par leur extrémité antérieure. La description de cette phase (forme, taille, noyau, etc...) coïncide tout à fait avec les stades intermédiaires que nous observons chez la grégarine parasite de *Glossobalanus minutus* dans une phase de croissance similaire. Il nous semble donc, que Théodorides et Desportes n'ont pas pu observer

les stades initiaux et ont consideré comme tels les stades intermédiaires.

Cette hypothèse est appuyée par le fait que les formes libres que nous observons dans la lumière des diverticules hépatiques atteignent une taille de 120–125 μm de longueur, dimensions que les auteurs donnent pour le trophozoïte libre.

Le nombre de stries longitudinales observables chez les gamontes est également un caractère utilisé pour la description de *Selenidium metchnikowi* et de *S. grassei*. Dans ce cas, nos observations coïncident avec celles de Théodoridès et Desportes. Nous distingons, en effet, 7–8 stries par individu, alors que Léger et Duboscq n'en différencient que 5.

D'autre part, si on considère l'existence d'une spécificité parasitaire, comme l'indique Ormières pour les Tuniciens et comme le suggèrent Théodoridès et Desportes, il serait logique de penser que la grégarine parasite que nous avons observée chez *Glossobalanus minutus* correspondrait à l'espèce décrite par Léger et Duboscq. Cependant, ceci n'a pu être établi, ayant donné les similitudes qui apparaissent avec *S. grassei* et les différences rencontrées avec *S. mechnikowi*.

Bien que les espèces de *Selenidium* o'u les phases de schizogonies restent inconnues soient nombreuses (entre autres, l'espèce type, *S. pendula* Giard, 1884), ainsi que la syzygie, le gamétokyste et le sporokyste (Tuzet et Ormières 1964, Levine 1974) et pour lesquelles les auteurs utilisent pour leur détermination des critères de taille et de forme des trophozoïtes, noyaux, stries longitudinales etc., nous considerons que ceux-ci sont insuffisants pour caractériser avec certitude l'espèce que nous avons rencontrée chez *Glossobalanus minutus*.

L'observation des différentes phases de la reproduction sexuelle de la grégarine, permettrait d'établir avec certitude qu'elle est l'espèce en question. Cependant, on doit faire mention de l'absence de ces phases dans toutes les coupes étudiées. Ceci pourrait être dû au lavage préalable auquel furent soumis les exemplaires afin d'éliminer le sable du tube digestif et pouvoir réussir aux différentes techniques histologiques.

Cependant, on peut considérer, comme le suggérait Ray (1930) que les formes sexuelles abandonnent le corps de l'hôte pour terminer leur développement dans le milieu extérieur ou dans le corps d'un autre animal, ou bien, comme le signale Levine (1974) qu'il existe une corrélation entre le cycle du parasite et celui de l'hôte ou avec les périodes de l'année.

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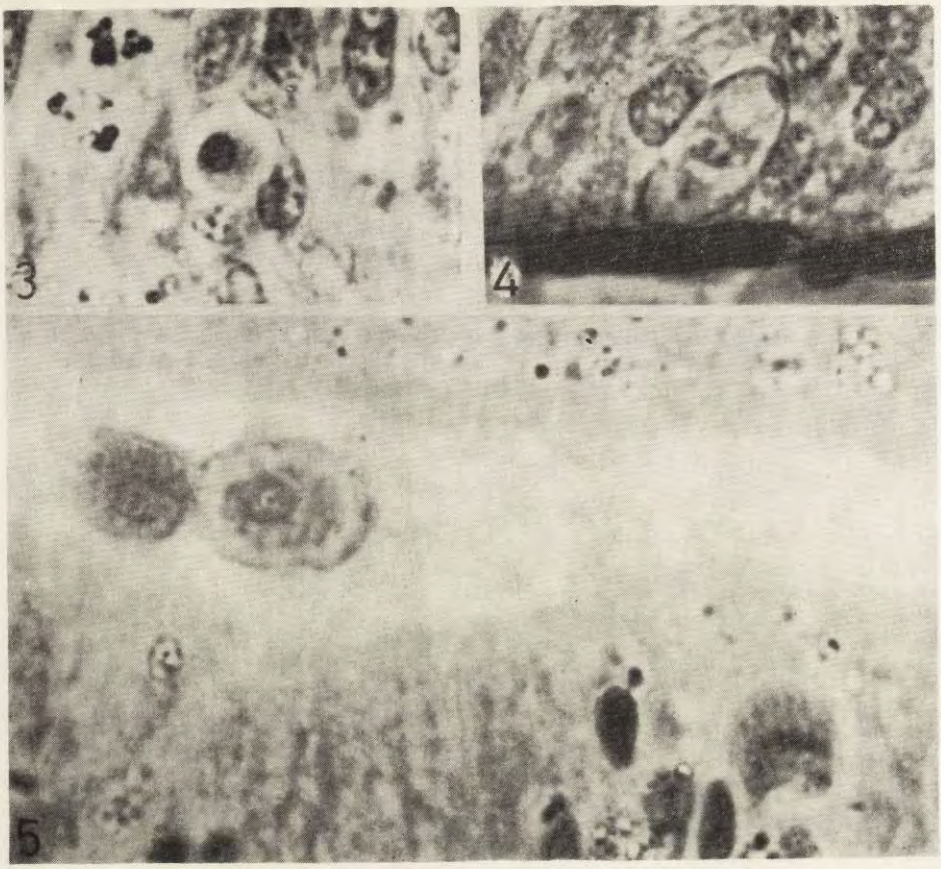
EXPLICATIONS DES PLANCHES I–II

PLANCHE I:

3: Jeune schizonte. Azocarmin de Heidenhain. (× 1800)
4: Schizonte dans la partie basale de l'épithélium. Azocarmin de Heidenhain. (× 1800)
5: Stade finale de la Schizogonie ou "barrilet" et section de la grégarine dans la lumière du diverticule. Pichrofuchsine de Van Gieson. (× 1800)

PLANCHE II:

6: Jeune gamonte à la couche ciliée de l'épithélium. Pichrofuchsine de Van Gieson. (× 1800)
7: Emplacement du jeune gamonte dans la cellule épithéliale. Bleu de Toluidine. (× 1800)
8–10: Plusieurs stades du développment de la grégarine: 8 — Bleu de Toluidine. (× 1800), 9 — Pichrofuchsine de Van Gieson. (× 1800), 10 — DNFB Tetrazo. (× 1800)
11: Partie antérieure du gamonte "en mucron". Detail. Ferricyanure ferrique de Adams. (× 1800)
12: Grégarine dans la lumière du diverticule hépatique. Azocarmin de Heidenhain. (× 720)
13: Partie postérieure de la grégarine. Detail de la figure précédente. Azocarmin de Heidenhain. (× 1800)



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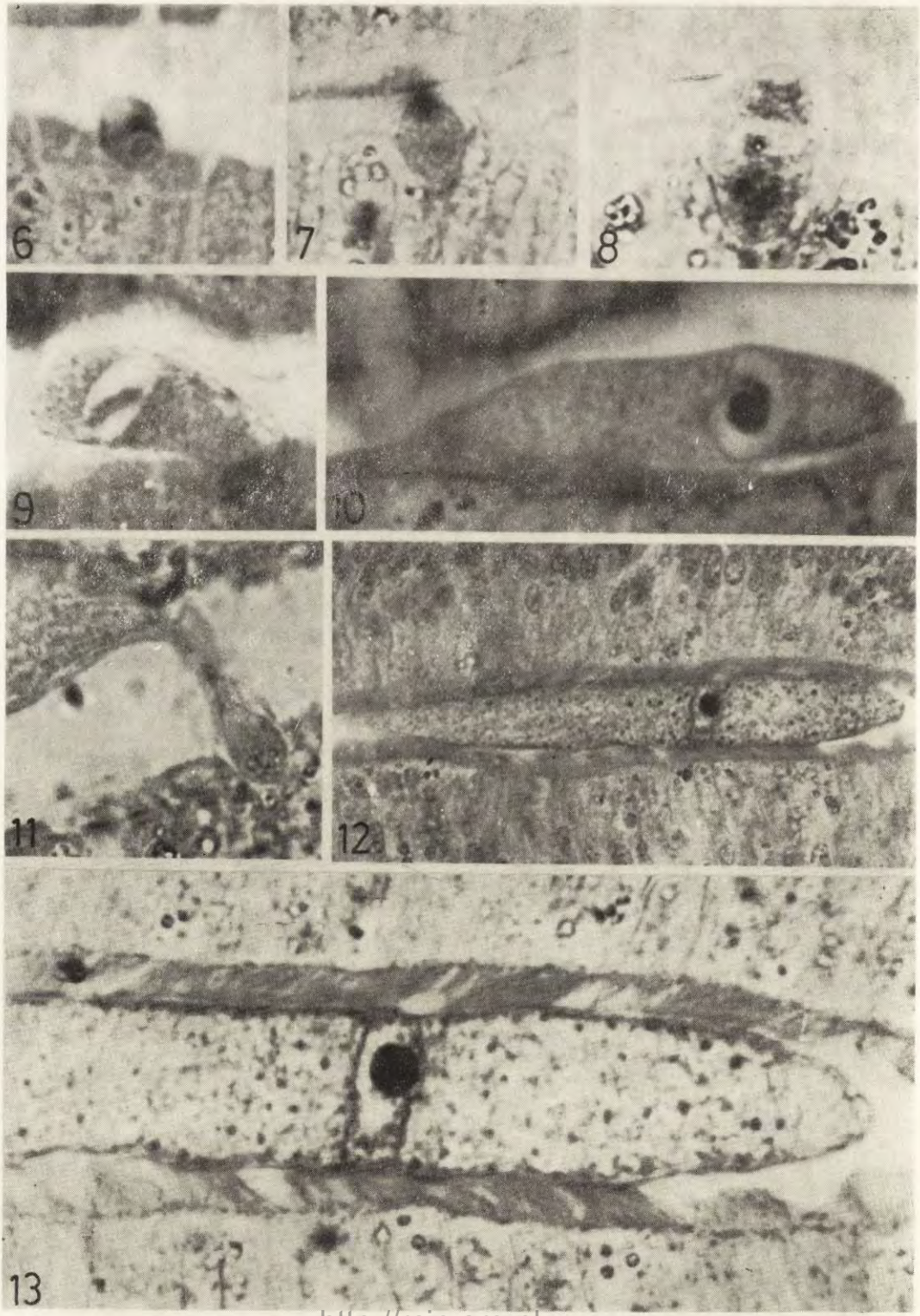

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On Three New Myxosporidian Parasites (*Myxozoa*) of the Ophicephalid Fishes of West Bengal, India

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Synopsis. The description of three new species of myxosporidian parasites (*Myxozoa*) from the gall bladder of some ophicephalid fishes of West Bengal, India is given. These are *Chloromyxum meglitschi* sp. n. from *Ophicephalus punctatus* Bloch., *Myxidium striatusi* sp. n. and *Myxosoma noblei* sp. n. from *Ophicephalus striatus* Bloch. The characteristic spore dimensions of the respective myxosporidians are 7.9 μm × 7.0 μm, 14.53 μm × 5.61 μm and 12.7 μm × 9.5 μm.

Since Chakravarty (1939) ophicephalid fishes have been extensively investigated for their myxosporidian parasites and altogether fourteen species under five genera viz. *Henneguya* Thelohan (3 spp.), *Myxobolus* Bütschli (3 spp.), *Myxosoma* Thelohan (2 spp.), *Unicauda* Davis (5 spp.) and *Zschokkella* Auerbach (1 sp.) have so far been described by Chakravarty (1939), Tripathi (1951), Bhatt and Siddiqui (1964), Lalitakumari (1965, 1969), Ray Chowdhury and Chakravarty (1970), Narasimhamurti and Kalavati (1975), Kalavati et al. (1981) and Kalavati and Narasimhamurti (1981). In this paper, three more species have been described under the genera viz. *Chloromyxum* Mingazzini, *Myxidium* Bütschli and *Myxosoma* Thelohan from the gall bladder of some ophicephalid fishes.

Materials and Methods

All the examinations of tissues were performed from live fishes collected from the tanks of Chinsurah and North-24-Parganas of West Bengal, India. The parasites were studied from fresh wet materials as well as from the dry smears

stained with Giemsa after fixation in absolute methanol. The fresh spores were treated with freshly prepared Lugol's iodine solution for the ditection of iodinophilous vacuoles and were also subjected to India-ink technique (Lom and Vavra 1963) for the ditection of mucus envelop. The extrusion of the polar filaments was achieved with 2.5% Potassium hydroxide solution. A prism type camera lucida was used for making the illustrations. The measurements were taken in micrometer (μm).

Observations

Chloromyxum meglitschi sp. n.

Description:

Trophozoite — coelozoic, ellipsoidal, disporous with granular cytoplasm (Fig. 1 *1*).

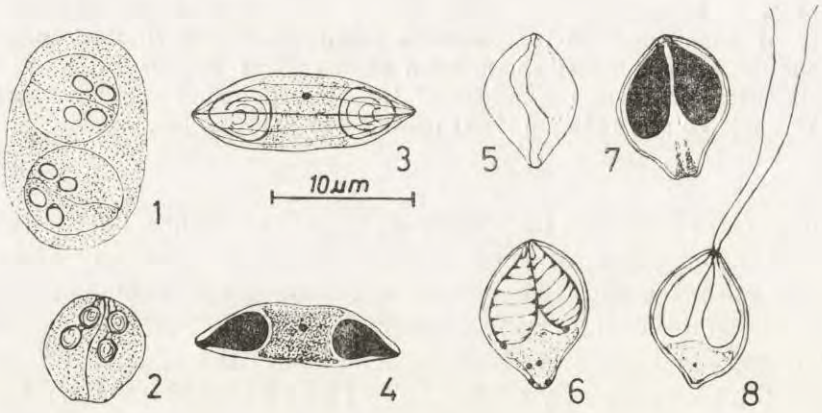

Fig. 1 *1–2*. Camera lucida drawing of *Chloromyxum meglitschi* sp. n. *1* — A disporous trophozoite — fresh, *2* — A spore — treated with Lugol's iodine, *3–4* — Camera lucida drawing of *Myxidium striatusi* sp. n. *3* — A spore in sutural view — treated with Lugol's iodine, *4* — The spore in valvular view — stained with Giemsa, *5–8*. Camera lucida drawing of *Myxosoma noblei* sp. n. *5* — A spore in sutural view — fresh, *6* — A spore (immature) in valvular view showing six nuclei — fresh spore treated with Lugol's iodine, *7* — A spore showing posterior extension of shell valve in valvular view — stained with Giemsa, *8* — A spore showing a part of its extruded polar filament — fresh spore treated with 2.5% KOH solution

Cyst — unknown.

Spore — coelozoic, small, ovoidal with truncated narrower end, suture slightly curved, shell valve smooth, polar capsule four, ovoid to pyriform, situated towards the broader end of the spore, open through

small narrow duct, sporoplasm with fine granules and uniformly dispersed, nucleus indistinct, iodinophilous vacuole not seen (Fig. 1 *2*).

Dimensions (based on the measurements of 7 trophozoites and 29 spores):

Length of trophozoite	15.36	(11.0–16.5)
Breadth of trophozoite	12.0	(9.0–13.0)
Length of spore (sutural diameter)	7.9	(7.0–8.5)
Breadth of spore	7.0	(6.0–8.0)
Length of polar capsule	2.7	(2.0–3.5)
Breadth of polar capsule	2.0	(2.0–2.5)

Host: *Ophicephalus punctatus* Bloch.

Infection locus: Gall bladder (trophozoites and spores found floating in the bile).

Incidence: 2 infected out of 10 examined showing heavy infection in both.

Locality: North-24-Parganas, West Bengal, India.

Remarks: Amongst the *Chloromyxum* spp., the present form resembles *C. levigatum* Jameson, 1931, *C. amphipnoui* Ray, 1933 and *C. kabatai* Moser and Noble, 1977 in the shape of the spore. However, the ovoid to pyriform polar capsules of the present myxosporidian differs from the long and narrow polar capsules of *C. levigatum*. It also differs from *C. amphipnoui* in having the smaller dimensions of the spore and polar capsules (the dimensions of the spore and polar capsules of *C. amphipnoui* are 8.24 × 10.3 and 4.1–5.2 × 3.1–4.0 respectively). The spore of *C. kabatai* is also much larger (16.9 × 16.0) than that of the present species. Moreover, the present myxosporidian differs from all the three *Chloromyxum* spp. by its truncated narrower end. I, therefore, consider the parasite new and the name *Chloromyxum meglitschi* sp. n. is given after Prof. Dr. P. A. Meglitsch of the Drake University, Iowa.

Myxidium striatusi sp. n.

Description:

Trophozoite and cyst — not found.

Spore (Fig. 1 *3* and *4*) — coelozoic, cylindro-biconical in sutural view, bilobate in valvular view due to the depression around the intercapsular region, suture straight, shell valve with fine longitudinal (end to end) striations, polar capsule two, pyriform, open almost vertically to the sutural line, sporoplasm uninucleated, finely granulated, com-

pletely filling the extra-capsular cavity, iodinophilous vacuole not seen.

Dimensions (based on the measurements of 24 spores):

Length of spore (sutural length)	14.53 (11.1–18.7)
Breadth of spore	5.61 (4.7–7.0)
Length of polar capsule	4.47 (3.7–5.6)
Breadth of polar capsule	3.04 (2.8–3.7)

Host: *Ophicephalus striatus* Bloch.
Infection locus: Gall bladder (bile)
Incidence: 4 infected out of 26 examined (infection scanty)
Locality: Chinsurah, West Bengal, India

Remarks: Of the many *Myxidium* spp. known today (Kudo 1933, 1934, Meglitsch 1937, Chakravarty 1939, 1943, Schulman 1962, Noble 1966, Iversen et al. 1971 and Moser et al. 1976), the present species closely resembles *Myxidium heteropneustesi* Chakravarty, 1943 in the dimensions of the spore. However, its bilobate spore in valvular view is markedly different from that of *M. heteropneustesi*. Moreover, it seems to be the first report from any ophicephalid fish. In view of such differences, the present myxosporidian has been considered a new species for which the name *Myxidium striatusi* sp. n. is given after the specific name of the host.

Myxosoma noblei sp. n.

Description:

Trophozoite — not found.

Cyst — oval, 121.0 × 98.0 (largest), found on the inner wall of the gall bladder.

Spore (Fig. 1 *5–8*) — nearly lenticular in sutural view, suture thick, ridged and almost S-shaped (Fig. 1 *5*), broadly elliptical in valvular view with short posterior extension of the shell valve which appears blunt, round or slightly pointed (Fig. 1 *6–8*), shell valve horizontally striated, spore wall thick with mucus envelope, polar capsule two, pyriform, equal and convergent, however, the two ducts open very closely but never cross, seven to eight coils of polar filament in each capsule, longest extruded polar filament 76 μm, sporoplasm granular, uninucleate or binucleate filling the small extracapsular cavity, iodinophilous vacuole absent, found floating in the bile.

Dimensions (based on the measurements of 40 spores):

Length of spore (sutural diameter)	12.7 (11.5–14.3)
Breadth of spore	9.5 (8.3–10.5)
Length of polar capsule	7.0 (5.8–8.5)
Breadth of polar capsule	3.7 (3.0–4.5)

Host: *Ophicephalus striatus* Bloch.

Infection locus: Gall bladder (cysts found on the inner wall of gall bladder and free spore found floating in the bile)

Incidence: 10 infected out of 26 examined (infection scanty except in 3 hosts where moderate infection noticed).

Locality: Chinsurah, West Bengal, India.

Remarks: Of the two species of *Myxosoma* Thelohan viz. *M. andhrae* Lalitakumari, 1969 and *M. channai* Kalavati et al. 1981 described from the ophicephalid fishes of India, the present species shows similarity with *M. andhrae* in having a posterior appendage of the spore. It also resembles *Myxobolus latipinnacola* Wold and Iversen, 1978 as its cysts were found on the inner wall of *Poecilia latipinna* (Lesueur). However, the myxosporidian in study differs from *M. andhrae* in having shorter but wider spore (spore of *M. andhrae* is 12.1–15.7 × 5.7–8.6) and different infection locus (infection locus of *M. andhrae* is the outer wall of intestine). The present form also differs from *Myxobolus latipinnacola* by the absence of iodinophilous vacuole in the sporoplasm, shorter but wider spore and much longer polar filament (spore and polar filament of *M. latipinnacola* are 13.1 × 8.6 and 17.3). The present species is, therefore, considered new for which the name *Myxosoma noblei* sp. n. is proposed after Prof. Dr. E. R. Noble of the University of California.

Material: Syntype specimens of *Chloromyxum meglitschi* sp. n., *Myxidium striatusi* sp. n. and *Myxosoma noblei* sp. n. on slides No. CON — 21, MOC — 5 and MXC — 32 respectively have been deposited at present in the Department of Zoology, R. B. C. College and will shortly be sent to the Zoological Survey of India, Calcutta.

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Myxosoma ganguli sp. n. (*Myxozoa: Myxosomatidae*) from the Head Cartilage of *Sillago maculata Quoy* and Gaimard (*Sillaginidae*)

Received on 4 April 1982

Synopsis. A myxosporidian parasite *Myxosoma ganguli* sp. n. (*Myxozoa* : *Myxosomatidae*) has been described from the head cartilage of a marine teleost *Sillago maculata* Quoy and Gaimard (*Sillaginidae*), caught in the shallow sea-water of South Andaman, India. The dimensions of its spores are 8.7 μm × 5.0 μm × 4.6 μm and it has been compared with the other *Myxosoma* spp. reported from the cartilage of other fishes.

The knowledge about the myxosporidian parasites of the teleost fishes of Indian sea-water is very scanty. Only a few species have so far been reported from the marine teleosts of India by Ganapati (1941) and Narasimhamurti and Kalavati (1979). Recently, three more species have been reported by Sarkar and Mazumder (1982). This paper records the description of a new myxosporidian from the head cartilage of a marine fish collected by the second author from Andaman Islands, India.

Material and Methods

A few fish were captured from the sea coast (about 1.5 to 2.0 m depth) of South Andaman in October, 1981. Eight of the formalin-fixed (10%) fish were brought to the laboratory and every part of the fish was examined under the binocular (dissecting and compound) microscopes. The parasite was studied from wet smears as well as from the dry smears stained with Giemsa after fixation in absolute methanol. For the ditection of iodinophilous vacuole, the method employed by Moser and Noble (1977) was followed. A prism type camera lu-

cida was used for making the illustrations. All the measurements with mean and range within parenthesis were taken in micrometer (μm).

Observations

Myxosoma gangulii sp. n.

Description:

Cyst — not found.

Trophozoite — ovoidal, about 100 μm in dimension, pansporoblasts spherical, 11 μm to 14 μm in diameter with 1 to 4 spores, disporous and tetrasporous forms mostly found.

Spore — suture thick, ridged and oblique (Fig. 1 *1*), spore ovoidal to ellipsoidal in valvular view (Fig. 1 *2*) with slightly pointed capsular

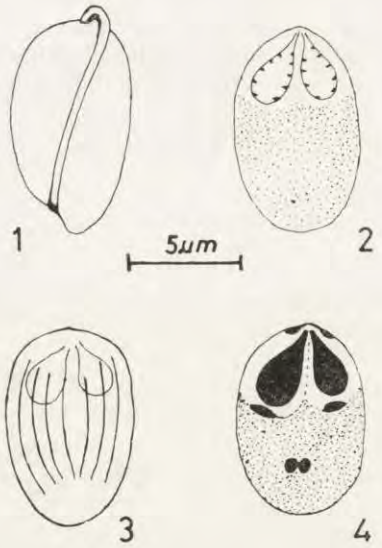

Fig. 1 *1–4*. Camera lucida drawing of *Myxosoma gangulii* sp. n., *1* — An unstained spore in oblique view showing thick and ridged suture, *2* — A spore in valvular view treated with Lugol's iodine, *3* — A spore in valvular view showing longitudinal striations on the shell valve, *4* — An immature spore in valvular view showing six nuclei — stained with Giemsa

and round posterior ends, shell valve with 6 to 8 longitudinal striations (Fig. 1 *3*), polar capsules 2 and unequal, pyriform and convergent, 5 to 6 coils of polar filament in each capsule, sporoplasm finely granulated and binucleate filling the small extracapsular space, iodinophilous vacuole absent, in immature spores (Fig. 1 *4*), 6 nuclei found of which 2 small valvogenous nuclei at the tip of the capsules, 2 larger capsulogenous nuclei at the base of the capsules and 2 small round nuclei in the sporoplasm.

Measurements (based on 48 formaline-fixed spores),

Spore

Length — 8.73 (8.0–10.0), Breadth — 5.03 (4.8–6.5),

Thickness — 4.6 (3.5–5.5),

Polar capsule (larger)

Length — 3.6 (2.8–4.6), Breadth — 1.9 (1.5–2.5),

Polar capsule (smaller)

Length — 2.4 (2.2–3.0), Breadth — 1.5 (1.3–2.0).

Infection locus: Cartilage of the head skeleton (posterio-ventral cranium).

Prevalence: One infected out of 8 fish examined, in October 1981.

Pathogenicity: Not apparent.

Host: *Sillago maculata* Quoy and Gaimard (*Sillaginidae*).

Locality: Rutland, South Andaman, India.

Material: Syntype specimens on slide No. ANMX — 12, kept in the Dept. of Zoology of this College, will shortly be submitted to the national collection of the Zoological Survey of India, Calcutta.

Discussion

Five *Myxosoma* spp. have so far been reported from the cartilage of various fishes (Table 1). Amongst them, *M. cerebralis* (Hofer) (cited from Lom and Hoffman 1971), *M. cartilaginis* Hoffman et al. 1965 and *M. filamentosa* Haldar et al. 1981 have been reported from the cartilage of head skeleton and *M. scleroperca* Guilford, 1963 [1] and *M. hoffmani* Meglitsch, 1963 have been reported from the cartilage of the sclera of the eye. A comparison of the present species with *Myxosoma* spp. mentioned above, shows its close resemblences with *M. cerebralis* (Hofer) in the length of the spore and in the number of coils of polar filament in each capsule. However, the spore of *M. cerebralis* (Hofer) is much wider and has larger polar capsules than in the present species. The comparison also reveals its similarity with *M. scleroperca* in having two unequal polar capsules in both, but the present myxosporidian differs from the latter and also from the other *Myxosoma* spp. by the smaller dimension of its spore. We therefore, consider this parasite a new species and the name *Myxosoma gangulii* sp. n. is proposed after Prof. Dr. D. N. Ganguly, the late Professor in Zoology of the University of Calcutta.

[1] Cited from Hoffman et al. 1965.

Table 1

Comparative spore dimensions of *Myxosoma* spp. reported from the cartilage of fishes

Specification	*M. cerebralis* (Hofer)	*M. cartilaginis* Hoffman et al.	*M. filamentosa* Haldar et al.	*M. hoffmani* Meglitsch	*M. scleroperca* Guilford	*M. gangulii* sp. n.
Spore						
Length	8.7 (7.4–9.7)	10.8 (10.0–12.0)	13.7 (11.2–17.3)	9.3 (8.6–10.8)	10.0–19.2	8.73 (8.0–10.0)
Width	8.2 (7.0–10.0)	9.5 (9.0–11.0)	9.5 (8.1–12.2)	8.4 (7.8–8.9)	7.2–9.6	5.03 (4.8–6.5)
Thickness	6.3 (6.2–7.4)	6.1 (6.0–7.0)		6.2 (5.9–6.5)	6.0–9.6	4.6 (3.5–5.5)
Polar capsule	equal	equal	equal	equal	unequal	unequal
Length	5.1 (5.0–6.0)	5.3 (5.0–6.0)	5.8 (4.0–7.1)	5.0 (4.6–5.7)	10.8 or 9.5	3.6 (2.8–4.6) or 2.4 (2.2–3.0)
Width	3.2 (3.0–3.5)	3.1 (3.0–4.0)	3.1 (2.0–4.0)	2.4 (2.2–2.7)	2.4 or 3.6	1.9 (1.5–2.5) or 1.5 (1.3–2.0)
Polar filament	5 or 6 coils	5 to 7 coils	5 to 6 coils	10 coils (max)	—	5 to 6 coils
Infection locus	Cartilage of head skeleton	Cartilage of head skeleton and gill arch	Cartilage of head skeleton and menings	Cartilage of sclera of eye	Cartilage of sclera of eye	Cartilage of head skeleton
Reference	Lom and Hoffman (1971)	Hoffman et al. (1965)	Haldar et al. (1981)	Meglitsch, (1963)	Hoffman et al. (1965)	Present study

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A New Microsporidian *Nosema valamugili* from the Gut Epithelium of an Estuarine Fish, *Valamugil* sp.

Received on 28 May 1982

Synopsis. The morphology and life-history of a new microsporidian parasite *Nosema valamugili* sp. n. parasitic in the gut of *Valamugil* sp. is given. Cysts were spherical or oval and appeared as white granules on the intestine and measured 150–250 μm in diameter. They appear to have been formed from a single cell and have a thin limiting membrane. Spores were pyriform measuring 3.0–3.2 × 5.4–6.2 μm in the fresh condition. A dot-like PAS positive polar cap is present at the anterior end. Another PAS positive granule was seen by the side of the posterior vacuole. The granule was present even after the extrusion of the polar filament. The polar filament was wavy and uniformly thin and measured 80–100 μm in length.

Studies on microsporidian parasites of invertebrates and vertebrates in India are few. Kudo (1929) reported two microsporidians, *Thelohania indica* and *Thelohania obscura* from the indian mosquitoes. Subsequently Kalavati and Ganapati (1974), Kalavati (1976 a, b), Kalavati and Narasimhamurti (1976 a, b, 1977 a, b, 1978) and Narasimhamurti and Kalavati (1978) reported several species of microsporidians from different invertebrate hosts from India. Among the parasites of vertebrate hosts, only four species of microsporidians have so far been reported from India and all of them are from piscean hosts. They are *Pleistophora sauridae* and *Nosema sauridae* from the muscles of *Saurida tumbil* (Narasimhamurti and Kalavati 1972) and *Glugea nemipteri* and *Nosema bengalis* from the liver and gills of *Nemipterus japonicus* respectively (Weiser et al. 1981). In the following account the morphology and life-history of another microsporidian parasite from the gut epithelium of *Valamugil* sp. is given.

Material and Methods

Valamugil sp. was collected from the small canals forming estuaries near Visakhapatnam and Bheemunipatnam and were examined for microsporidian parasites. Smears were prepared from the small cysts, fixed and stained using the conventional methods. Bits of the infected intestine were fixed in alcoholic Bouin's or Carnoy' fluid, sectioned at 8 μm thickness and stained using the usual histological stains. Spores were examined in the fresh condition, both under the bright field and dark ground illumination. The spores were either stained with Giemsa or Heidenhain's iron haematoxylin or treated according to the Feulgen's technique. PAS technique was used to demonstrate the polar cap. Spores were hydrolysed in 1 N HCl at 60°C for 10 min prior to staining with Giemsa or Heidenhain's iron haematoxylin.

Observations

250 *Valamugil* sp. were examined for microsporidian parasites during 1980–1981. There were no external indications of infection and hence all the fish had to be dissected before infection was noticed. Infection was noticed during February–April and only 2% of the fish examined revealed infection. The intensity of infection was low and 10–15 cysts only were recovered from a single infected host. Cysts were spherical or oval and appeared as white granules on the intestine when observed under a binocular microscope. The cysts were always observed in the outer epithelial cell layer and measured 150–250 μm in diameter. The cysts appear to have been formed from a single cell and have a very thin limiting membrane. Infected cells were hypertrophied and the host cell nuclei were pushed to a corner of the cell. The entire spore mass was found in a vacuole inside the host cell (Fig. 1 *1*). The vegetative stages of development of the parasite were clearly seen in sections treated according to the Feulgen technique. Schizonts were irregular in shape having dense cytoplasm and contained a diplokarya. The chromatin was in the form of a bilobed mass and was surrounded by a clear space and a fine membrane. The earliest binucleate stage observed measured 3.4–4.2 μm and was irregular in shape having an incipient cell wall (Fig. 1 *2*). Mature schizonts were always ribbon-like and had a well defined wall. They measured 16.5–18.0 × 3.2–4.6 μm and showed vacuolated cytoplasm and as many as 16 paired nuclei. This was followed by condensation of the cytoplasm around the nuclei which was probably the beginning of the sporogonial sequence.

Sporonts were either oval or bean-shaped measuring 5.2–6.4 × 9.0–10.5 μm with 2 or 4 nuclei arranged in pairs (Fig. 1 *4* and *5*). The

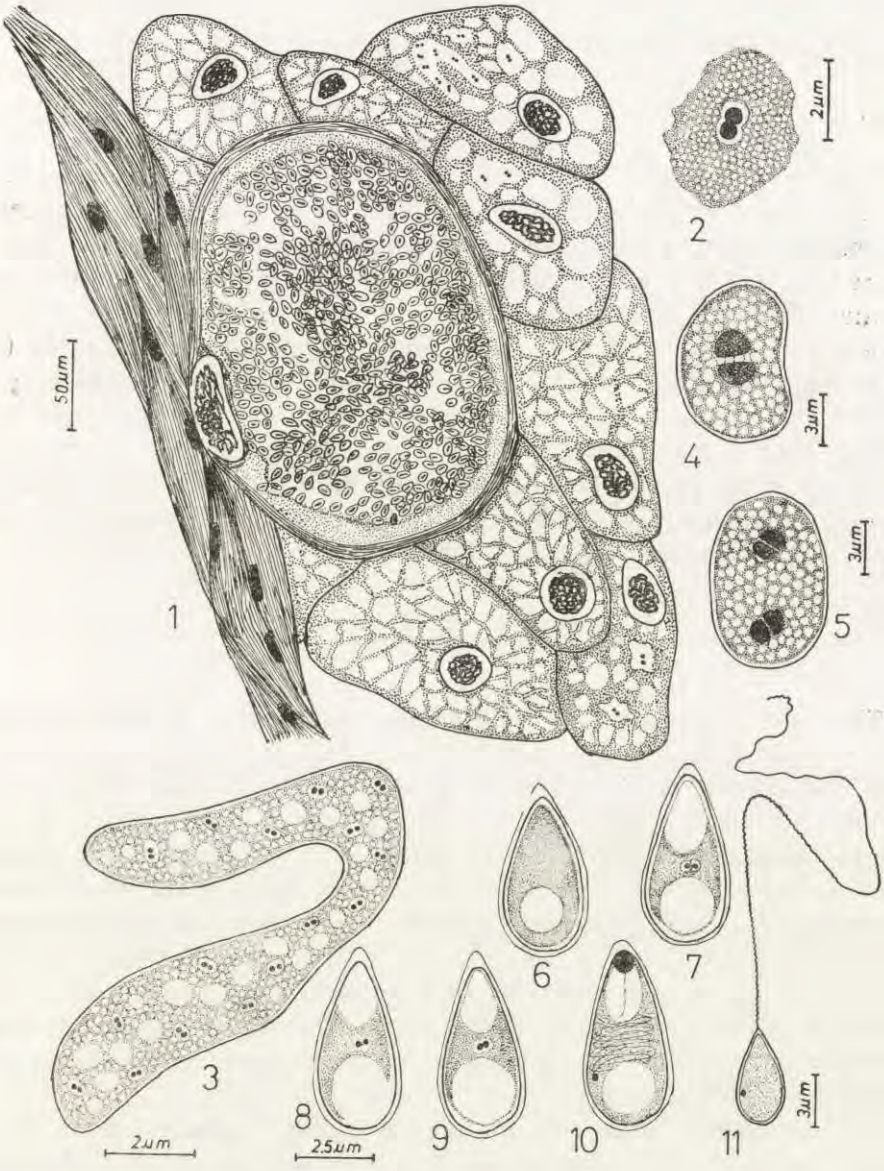

Fig. 1. *1* — Section of the cyst, *2* — A schizont, *3* — A multinucleate schizont, *4–5* — A binucleate and tetranucleate sporont, *6* — A fresh spore, *7* — A spore stained with Giemsa, *8* — A spore stained with Feulgen, *9* — A spore stained with iron haematoxylin, *10* — A spore stained with PAS, *11* — A spore with extruded polar filament

nucleus was deeply stained and the cytoplasm was lightly stained and coarsely alveolated. These stages were very few and were scattered amongst the spore masses.

Spores were pyriform measuring 3.0–3.2 × 5.4–6.2 μm in the fresh condition. The anterior end was pointed with a slight thickening and the posterior was rounded. A clear spherical vacuole was present near the posterior end (Fig. 1 *6*). The spore wall was thick and refractile. Spores stained either with Heidenhain's iron haematoxylin or Giemsa showed two minute spherical nuclei placed side by side in the sporoplasm (Fig. 1 7 and *8*). In Feulgen stained preparations a fine chromatin thread was seen extending between the two nuclei (Fig. 1 *9*). A spherical dot-like polar cap could be seen when stained according to the PAS technique. Another PAS positive granule was seen by the side of the posterior vacuole. The polar filament usually terminated near this granule (Fig. 1 *10*). The PAS positive granule was seen even after the extrusion of the polar filament. An addition of a drop of H_2O_2 released the polar filament in 40–50% of the cases. The polar filament was uniformly thin and measured 80–100 μm (Fig. 1 *11*).

Discussion

The two important and well demarcated genera of microsporidia so far reported from fishes are *Ichthyosporidium* Caullery and Mesnil, 1905 and *Glugea* Thelohan, 1895. Both the genera from conspicuous xenomas. A thick walled cyst is formed from a single hypertrophied cell in *Glugea* and a multicystic lesion with ramifications is formed in *Ichthyosporidium*. In the other two genera, *Nosema* and *Pleistophora* reported from fishes no specific xenomas are reported. It is not possible to assign the present form to either of the two genera, *Glugea* or *Ichthyosporidium* since it has a very thin membrane all round. However, it showed all the characters of the genus *Nosema* in having an apansporoblastic development, diplokarya in the developmental stages and binucleate spores.

Of the nearly 98 species of microsporidians reported from fishes 48 belong to the genus *Glugea*, 34 to *Pleistophora*, 2 to *Thelohania*, 1 each to *Spraguea* and *Mrazekia* and 12 belong to the collective group *Microsporidium* and only 2 valid species in the genus *Nosema* (Sprague and Vavra 1977). They are *Nosema* sp. Poljansky (1955) from *Mollotus villosus* and *Nosema* sp. (Lom 1972) from *Hippocampus erectus*. Both the species were incompletely described and hence it is not possible to compare them with the present from. Weiser

et al. (1981) described another species, *N. bengalis* from the gills of *Nemipterus japonicus* (ignoring the statement of Sprague and Vavra that the genus *Nosema* is an invertebrate genus). The cysts in *N. bengalis* were formed in migrating lymphocytes, spores were binucleate and have a narrow anterior end and a bulbous posterior region while in the present form the cysts were found in the hypertrophied epithelial cells of the intestine and the binucleate spores were typically pyriform. The spores of *N. bengalis* are smaller in size and measured 3.0–3.5 × 1.5–1.7 μm while in the present form they are 3.2–4.5 × 4.5–5.4 μm. The polar filament is typically wavy starting from a PAS positive granule. *Nosema sauridae* is another microsporidian described earlier from the visceral muscles of *Saurida tumbil* (Narasimhamurti and Kalavati 1972). Since the spores were uninucleate, Sprague (1977) transferred it to the collective group *Microsporidium*.

The present form differs from the other species of *Nosema* described from piscean hosts in the site of infection and in the size of the spores. Further this is the first report of a microsporidian from an estuarine fish and from the Indian subcontinent. Hence it is considered new to science for which the name *Nosema valamugili* sp. n. after the host is proposed.

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Histopathological and Experimental Infection Studies on *Pleistophora ganapatii* (Kalavati) a Microsporidian Parasite of *Odontotermes horni* (Desn.) [1]

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Synopsis. Observations were made on the histopathology of infection of *Pleistophora ganapatii* in the gut of *Odontotermes horni* (Desn.). Hypertrophy of the host cell, vacuolization of the host cell cytoplasm and the break down of the nuclear membrane were observed. Heavily infected cells ruptured releasing the spores into the lumen of the gut which blocks the passage of any material. The seasonal distribution of *P. ganapatii* showed that the maximum percentage of infection was 20 in the month of December and the minimum was in April being 6.6. Experimental infections showed the spores in the epithelial cells by about 96 h and all the experimental hosts died in 120 h. The nymphs were more susceptible to infection than the workers. The rate of mortality was high on the fourth and fifth days in the workers. The soldiers were not infected. *P. ganapatii* is host specific.

A number of investigators studied the effects of microsporidian parasites on insect hosts in the recent past and also carried out infection experiments to study the mortality rate. Kalavati (1976) has given an account of these studies.

In the present investigation an attempt has been made to study the pathogenicity of the microsporidian, *Pleistophora ganapatii* Kalavati parasitic in the gut of *Odontotermes horni* (Desn.). Experimental infection studies and the mortality rate were carried out and the results are presented in Tables 2, 3 and 4.

Material and Methods

Odontotermes horni (Desn.) were collected from different localities in and around Waltair (Andhra Pradesh, India) and were examined for microsporidian

[1] Abstract presented at the 16th International Congress of Entomology, Kyoto, Japan. August, 1980.

parasites. Two colonies which were widely separated, one having a high percentage of infected and the other having uninfected termites were chosen for the present study. Termites from the two selected colonies were repeatedly examined for over a period of three months to ascertain the presence or absence of infection prior to the commencement of the infection experiments.

The termites were collected by placing bits of moist filter paper in the two selected colonies for a few hours and later when the bits of filter paper were removed carefully a number of nymphs, workers and soldiers were seen crawling over their surface. The bits of filter paper were taken to the laboratory without causing any disturbance or injury to the termites. Batches of 25 each of the nymphs, soldiers and workers were transferred with the aid of a fine camel hair brush into separate petri dishes containing a layer of moist absorbent cotton wool at the bottom. A small quantity of fine saw dust, decaying wood and humus containing fungal material (collected from the vicinity of the colony where infection was absent) was provided as food material for the termites. They were maintained between 24–28°C and care was taken to see that the filter paper was always moist. The healthy termites were maintained under the above conditions for at least 48 h before feeding with the spores. Termites which were dead or which appeared lethargic either due to injury or some other reason were removed from the petri dishes.

The termites from the infected colony were similarly collected 1 h before the commencement of the experiment. They were decapitated the gut removed and examined microscopically for the presence of spores. When infection was present, the entire gut was placed on a slide in a drop of normal saline, covered with a cover slip and gentle pressure was applied when most of the spores from the gut escaped out. The spores thus released were washed into a small petri dish and concentrated by centrifugation at slow speed for about 15 min. The spores were then suspended in 5% glucose solution. Small quantities of the spore suspension was placed on bits of filter paper and kept in petri dishes for the termites to feed.

A second method adopted for infecting the termites with the spores was to place about 0.5 ml of spore suspension in a cavity slide and keeping the slide in a petri dish containing healthy termites. The petri dishes were placed in a battery jar containing moist cotton wool at the bottom and were covered with a lid. The termites gathered around the spore suspension and lapped it up. Batches of 25 healthy termites were always maintained as control under similar conditions.

After allowing the termites to feed for sometime they were transferred to fresh petri dishes. The termites were sacrificed at hourly intervals for the first six hours and later at intervals of 6 h and examined for infection by teasing out the guts on a slide and examining them under a microscope. In addition smears were also prepared in the usual manner, fixed in methyl alcohol and stained with Giemsa. Entire guts were also fixed in alcoholic Bouin's fluid, sectioned at 8 μm thickness and stained with Heidenhain's iron haematoxylin. All the termites from the control batch as well as from the experimentally infected lot were sectioned and examined for microsporidian infection. The mortality rate was recorded at six hourly intervals and the results are given in Tables 2 and 3.

Similarly infective feeding experiments were carried out on *Odontotermes obesus* (Rambur), *Coptotermes heimi* (Wasm.), *Macrotermes estherae* (Desn.) which

also occur in large numbers in and around the University Campus at Waltair, although in different habitats.

All the experiments were repeated at least thrice before any conclusions were drawn and proper controls were maintained in all cases.

Results and discussion

Histopathology. There were no external indications of infection. The infected guts appeared opaque milky white when seen under the low power of a compound microscope. A comparison of the sections of the infected and uninfected guts showed that the epithelial cells of the former were hypertrophied and the cytoplasm was highly vacuolated. The pansporoblasts and sporogonial plasmodia were seen in parasitophorous vacuoles. The host cell nuclei were hypertrophied followed by the break down of the nuclear membrane and release of the chromatin material into the surrounding cytoplasm in the form of small fragments. The cytoplasm was limited to the periphery leaving a large central portion filled with the spores.

Most of the workers showed heavy infection of epithelial cells which on bursting released the spores into the lumen of the gut. Large numbers of spores accumulated at the junction of the mid- and hind-guts in the region of the Malpighian tubules which blocked the passage of any material. In such cases the wall of the fore- and mid-guts was extensively damaged and the portion of the gut beyond the Malpighian tubules was attenuated.

Seasonal Variation. A total number of 6060 *O. horni* collected from two different localities in and around the University Campus were examined regularly to study the occurrence of *P. ganapatii* during 1978–1979.

The host termites occurred in large numbers after the onset of the monsoon and appeared on the surface areas during the month of Au-

Table 1

Seasonal variation and occurrence of *Pleistophora ganapatii* in *Odontotermes horni*

Months	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total
No. of *O. horni* examined	860	620	408	127	100	80	280	320	580	710	933	1042	6060
No. of *O. horni* infected	142	76	39	9	0	0	46	60	110	135	187	212	1001
Percentage infection	16.9	12.2	9.4	6.6	0	0	15.1	18.7	18.9	19.0	19.6	20.01	16.5

gust. They increased further and reached a maximum in December. The number of termites occurring on the surface were gradually reduced from February and reached a minimum in the summer months of April and May (Table 1). In the present study the data on the soldiers was not included since they were never infected with the microsporidian and as such for purposes of seasonal variation the workers and nymphs were alone taken into consideration.

Infection was present throughout most of the year (except May and June) and the termites showed large number of spores in their guts. The percentage infection was minimum in April being 6.6 and maximum in December being 20.0. The percentage gradually increased through the months with increasing availability of hosts (Fig. 1).

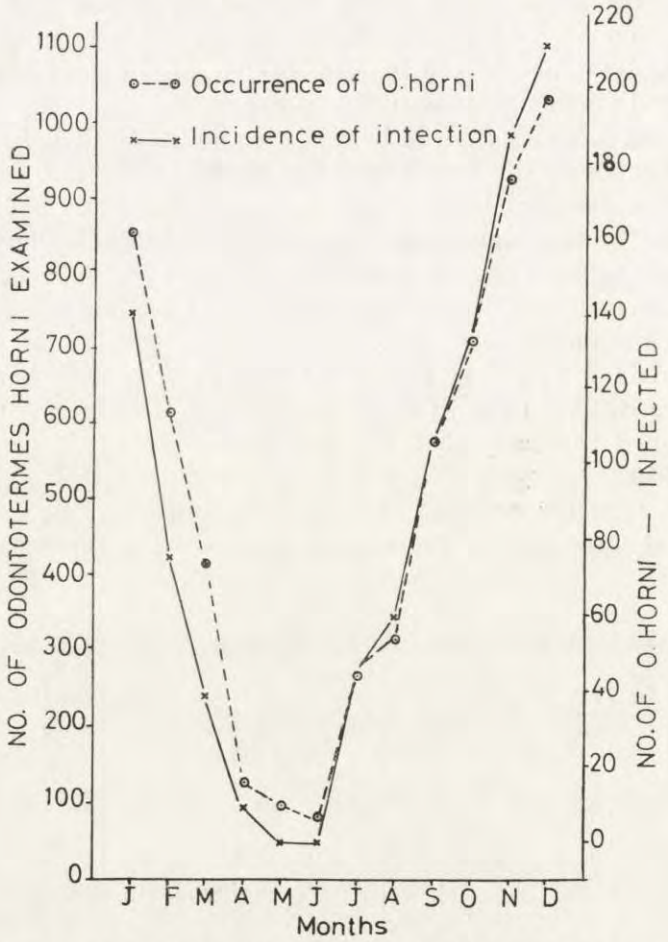

Fig. 1. Infection of *Odontotermes horni* by *Pleistophora ganapatii*

In experimental infection the rate of mortality was high and all the hosts died in course of time, while in natural infections the percentage mortality was not so high because a few infected termites could always be collected when the area was left undisturbed for some time.

The spores of *P. ganapatii* are known to lose their viability in 3 days at 37.5°C and die instantaneously at 40°C (Kalavati 1977). The average atmospheric temperature in the summer months of May and June in the area of investigation ranged between 37.0–40.0°C and hence the spores released from the infected hosts were probably becoming non viable and only such of those spores which were in the interior of the termatorium where the temperature was lower and was around 24.0°C survived which probably spread the infection again which starts in the months of July and August after the onset of the monsoon. Further the spores of *P. ganapatii* could not withstand dessication for a period of more than 3 h and apparently became non viable because the polar filaments in such cases could not be extruded by using the same methods. Probably dessication is also one of the factors which controlled the viability of the spores and spread of infection in nature.

Experimental Infection. Termites belonging to different genera and species obtained from three different habitats were used for experimental infection studies. Positive results were obtained only in termites belonging to one species. When the spores were fed to these termites they were seen in the region of the fore gut within 15 min after the infective feed and the polar filaments were released in about 50% of the spores. The distal portion of the filaments in some spores were seen anchored to the epithelial cells while the proximal portion was still coiled like a watch spring. A small portion of the polar filament (about 5.0 μm) was slightly bulged at different levels in different spores and was deeply stained probably because of the presence of sporoplasm.

A typical amoebula emerging out of the polar tube was not observed. Small bi- and tetra-nucleate schizonts measuring 3.0–4.0 μm and having small dot-like chromatin granules surrounded by a clear halo were seen in the epithelial cells in about 18 h after the infective feed. Similar stages were observed in sections also. There was no vacuolization of the host cell cytoplasm at this stage. After about 48 h of experimental infection the epithelial cells showed hypertrophy and vacuolated cytoplasm. By about the end of the third day multinucleate schizonts as well as bi- and tetra-nucleate sporonts were seen inside the vacuolated cytoplasm. The sporonts could be seen in smears stained

with Giemsa. About 80 h after the experimental infection the epithelial cells and their nuclei were nearly double their normal size. Subsequently the nuclear membrane of the host cell nucleus was broken down releasing the chromatin material into the surrounding cytoplasm. Smears prepared 86 h after the experimental feed showed sporogonial plasmodia measuring 17.6–19.8 × 14.8–16.2 μm and containing 16–28 nuclei. The epithelial cells were filled with developing sporoblasts and showed some stages of spore morphogenesis by the end of 96 h. The filament was released in about 20–25% of cases only, probably because the spore morphogenesis was not completed.

All the hosts in the experimental study died in about 120 h. Sections of the guts of such hosts showed that the epithelial cells were ruptured releasing the spores and the cell contents into the lumen of the gut which formed a solid mass blocking the lumen in the region of the Malpighian tubules. 90% of the spores released the polar filaments by adopting the usual methods indicating the maturation of the spores. The extensive damage of the gut epithelium on account of the infection and blocking of the lumen of the gut by the spores and cell exudates may probably be the cause of death of the hosts.

Rate of Mortality of the Nymphs and Workers. Three batches, of 25 each of healthy nymphs were given an infective feed with the spores of *P. ganapatii* as described earlier and were maintained in the laboratory. The rate of mortality was studied at regular intervals. In all the three batches it has been observed that the nymphs succumbed to the infection earlier than the workers. Death of the nymphs was first observed at the end of 24 h. In all the three batches of nymphs put together 8% died at the end of 24 h, 9% died at the end of 36 h and 20% died at the end of 48 h. 40% died on the third day, 19% died on the fourth day and 4% died on the fifth day (Table 2).

Table 2

Rate of mortality of the nymphs of O. *horni* infected with *P. ganapatii*

No.	Jars of 25 each	6 h	12 h	18 h	24 h	36 h	48 h	3rd day 72 h	4th day 96 h	5th day 120 h	6th day 144 h	7th day 168 h
(1)	A	—	—	—	4	3	7	9	2	—	—	—
(2)	B	—	—	—	0	3	4	11	6	1	0	0
(3)	C	—	—	—	2	1	4	10	6	2	0	0
(4)	D (control)	—	—	1	—	—	—	—	—	—	2	—

A microscopical examination of the guts showed large numbers of hypertrophied epithelial cells showing sporogonial plasmodia but not fully formed spores. Death was probably because of the damage to the epithelial cells caused by the heavy infection.

Similar experiments carried out on the workers showed that death was observed for the first time at the end of third day (10.7%) and the epithelial cells of the guts of such dead insects showed only sporogonial plasmodia. The epithelial cells were hypertrophied but not ruptured. The rate of mortality of workers during the next two days was high being 40% on the fourth day and 47% on the fifth day. Only a few (3%) continued through the sixth day when they also died (Table 3).

Table 3

Rate of mortality of the workers of *O. horni* infected with *P. ganapatii*

No.	Jars of 25 each	6 h	12 h	18 h	24 h	36 h	48 h	3rd day 72 h	4th day 96 h	5th day 120 h	6th day 144 h	7th day 168 h	8th day 192 h
(1)	A	—	—	—	—	—	—	4	8	13	—	—	—
(2)	B	—	—	—	—	—	—	3	12	10	—	—	—
(3)	C	—	—	—	—	—	—	1	10	12	2	—	—
(4)	D (Control)	—	1	—	—	—	—	—	—	—	1	—	—

All the dead insects showed pansporoblasts and spores filling the lumen of the gut. Control batches of 25 nymphs and 25 workers showed 1 or 2 deaths during the same period. They could be maintained for a period of 2 weeks more without any significant mortality.

Three batches of soldiers similarly infected did not pick up any infection and most of them survived for a period of 2 weeks. There were, however, a few deaths of soldiers during the period of observation but none of them showed any evidence of infection with the microsporidian parasite (Table 4).

Earlier authors in their studies on experimental infection of hosts showed that the time taken for the establishment of the parasite completion of the life-cycle and production of spores varies considerably depending on the age of the host, species of the parasite, size of the inoculum and other environmental factors (Weiser 1976).

In the present study it has been observed that the infection was initiated in about 18 h after the infective feed and histological changes were apparent in about 80 h after the infective feed by which time the

Table 4

Rate of mortality of the soldiers of *O. horni* infected with *P. ganapatii*

No.	Jars of 25 each	6h	12 h	18 h	24 h	36 h	48 h	3rd day 72 h	4th day 96 h	5th day 120 h	6th day 144 h	7th day 168 h	8th day 192 h
(1)	A	—	—	—	—	3	—	—	2	—	—	—	1
(2)	B	—	1	—	3	1	1	—	—	—	1	—	—
(3)	C	1	—	—	—	—	—	2	—	3	—	1	—
(4)	D (Control)	—	—	1	—	—	—	1	2	1	—	—	2

microsporidian has multiplied and formed the sporogonial plasmodia. Sporoblasts and spores were formed in about 96 h and by this time some of the experimental hosts succumbed to the infection. The rate of mortality was maximum on the fourth and fifth days by which time masses of spores were released into the lumen of the gut and the cells had frayed margins. The nymphs died earlier than the workers which shows that the former were more susceptible and succumbed to the infection more easily.

Cross Infection. Cross infection experiments were carried out on the termites collected from four different habitats with negative results. Infection could be established successfully only in the natural host, *Odontotermes horni* which is a xylophagous termite generally associated with decaying wood, other plant material and large quantities of humus. Infection experiments carried out on another xylophagous termite, *O. obesus* which attacks the bark of banana plants and also build earthen mounds. The humidity in these colonies was low as also the moisture content. The mounds were exposed to the sunlight and were generally dry except in the subterranean region. The spores fed to these termites were found in the lumen of the gut within 15 min and the empty spore shells were found in the faecal contents in about 2 h. The termites were maintained in the laboratory for 1 week but no further changes were observed. Apparently although the spores were able to germinate in the gut further development could not take place probably because of the non specific nature of the host. *Coptotermes heimi* was another termite which was experimentally infected with the spores of *P. ganapatii*. The termites were collected from underneath the dry leaves of the cashew nut plants in the sandy soil where they lived without much of moisture and humidity. The spores fed to them along with the food material were passed out along with the faeces in about 2.5 h time without any change. These spores were apparently

still viable since the polar filament could be extruded in 60–70% of the spores by using the usual methods.

Attempts to feed the non xylophagous termite, *Macrotermes estherae* with the spores of *P. ganapatii* were unsuccessful.

The above results reveal that the microsporidian, *P. ganapatii* is host specific unlike some of the other insect microsporidians which were infectious to a variety of hosts (Machay 1957, Kaya 1973 and Undeen and Maddox 1973). This may be due to several reasons as mentioned by Weiser (1976) and Tanada (1976).

The presence of high humidity and moisture and possible low temperature of the subterranean regions of the termatoria are all probably responsible for the survival of the spores for a longer time outside the host and subsequent establishment in the host. Weiser (1964) also stated that most protozoans survive at high temperatures but they cannot stand dessication as water vapour passes through the proteinaceous material which closes the polar opening of the spore. The individual host enzymes could also play a role in the failure of the establishment of the infection.

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Papers are accepted in English, French, German and Russian. Every paper should begin with the name and postal address of the laboratory name and the surname of the author and title in the language of the text. The titles in German and French should be translated into English according to the demands of Current Contents. The paper should be accompanied by synopsis in the language of the text not exceeding 100 words. In the Russian text also the name and the postal address of the laboratory, legends of tables, plates and text illustrations must be translated, the translation of the summary may be somewhat more extensive, and the name of the author should be given additionally also in the Latin characters.

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Gelei J. von 1939: Das aussere Stutzgerustsystem des Parameciumkörpers. Arch. Protistenk., 92, 245–272.

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The following material should be supplied on separate sheets 1. the running title for the page headlines, 2. tables, 3. legends for text figures, 4. legends for plates. Line-drawings will be published in the text, photographs and raster figures on separate plates. No coloured photographs can be published presently. Lettering on photographs and drawings should be marked in pencil. With no regard to the language of the text, only the Latin lettering, arabic numerals or generally accepted symbols are admittable for marking on illustrations. Numbering of text-figures, plates and tables must be also marked in pencil, as well as in the legends as in the text.

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In preparation

A. Grębecki and J. Kołodziejczyk: Contraction and Streaming Relations Recorded Simultaneously at Two Points Along the Plasmodial Veins and Frontal Channels of *Physarum polycephalum* — J. Kołodziejczyk and A. Grębecki: Effects of White-red Illumination Changes on the Coordination of Some Motor Functions in Plasmodia of *Physarum polycephalum* — W. Krawczyńska: Regeneration of *Tetrahymena* Cilia Under the Influence of a DNA-ligand: 4,6-diamidine-2-phenylindole (DAPI) — D. K. Chatterjee: Life Cycle of *Trypanosoma avium bakeri* and Its Host-specificity — I. Wita and K. M. Sukhanova: Studies on the Biology and Cytology of *Parastasia fennica* (Michajłow) (*Flagellata, Euglenida*), a Parasite of the Intestine of *Cyclopidae* (Copepoda) — A. C. Majewska and W. Kasprzak: The Rat as an Animal Model of *Giardiasis* — N. Gupta and D. S. Jairajpuri: *Trypanosoma batrachi* Qadri, 1962 and Its Effect on the Biochemical Composition of the Blood of *Clarias batrachus* — K. P. Janardanan and P. Ramachandran: On a New Septate Gregarine, *Stenoductus polydesmi* sp. n. (*Sporozoa : Cephalina*) from the Millipede, *Polydesmus* sp. with Observations on Host-specificity of Gregarines Infecting the Millipedes in Kerala, India

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