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Studies on the representatives of the family *Paraisotrichidae* Da Cunha (*Ciliata, Trichostomata*). II. Buccal infraciliature in the genus *Paraisotricha* Fior. and *Rhizotricha* Wolska

Badania nad przedstawicielami rodziny *Paraisotrichidae* Da Cunha (*Ciliata, Trichostomata*). II. Infraciliatura gębowa rodzaju *Paraisotricha* Fior. i *Rhizotricha* Wolska

In the preceding article (Wolska 1964) the somatic infraciliature of the species *Paraisotricha colpoidea* Fiorentini, *P. minuta* Hsiung and *Rhizotricha beckeri* (Hsiung) Wolska was investigated. Presently the oral ciliature of both *Paraisotricha* species will be discussed jointly as constructed in the same pattern. The photomicrographs concern mostly *P. minuta* because the silver impregnation appears in this species more distinct than in *P. colpoidea*. The oral apparatus of *Rhizotricha beckeri*, representing the genus *Rhizotricha*, will be discussed separately.

Paraisotricha Fiorentini

In the genus *Paraisotricha*, the peristome is an extensive cavity with a gradual slops behind the area of frontal cilia, descending inside the cytoplasm along the body axis somewhat towards the dorsal side. The buccal overture is limited on the right side by the margin with the paraoral kinety — UM. The right margin bends and continues as the ventral margin. That is initially free and bears a collar on its subsequent part (cf. Wolska 1964), rising anteriorly and to the left side, where the delimitation from the peristomal cavity and from the remaining body is far less distinct. Descending inside the cell body, the peristomal groove is narrowing gradually assuming the shape of a cone. In the cross section at the level of the half of the length, the lumen of the peristome is approximately triangular; the basis of the triangle corresponds more or less to the right wall, the arms meeting at a sharp angle correspond to the ventral and dorsal peristome walls. The configuration just described is represented in the scheme (Fig. 1) and in Pl. I 1—4, V 38.

The peristome ciliature consists in the bending ciliary rows of the collar. The kineties of the left collar side (left side of the ciliate) bend only slightly because the peristome itself is flat in this region, subsequently they pass over a slight convexity on the left margin, and at last smoothen — gradually

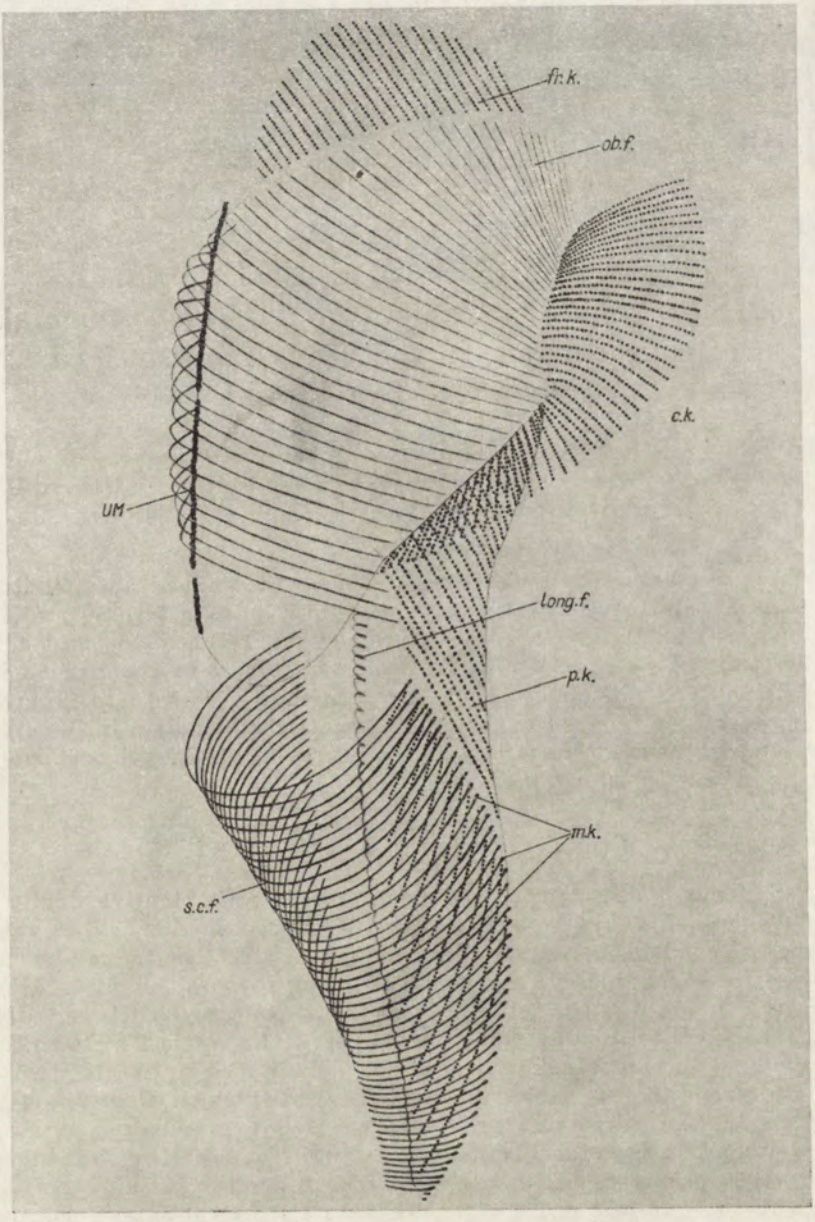

Fig. 1. Peristome in the genus *Paraisotricha* Fiorentini
fr.k. — frontal kineties, c.k. — collar kineties, long. f. — longitudinal fiber, p.k. — peristomal kineties, m.k. — marginal kineties, s.c.f. — semicircular fibers, UM — undulating membrane

approaching the area of frontal cilia (Pl. I 3). The distinct bending of kineties on the sharp ventral edge of the peristome begins approximately at the half length of the collar (Pl. I 5—6). Beginning from this place, the kineties which bend towards the peristome become gradually longer, and the corresponding segment of the collar — shorter. The peristomal kineties occupying the most right position (4 or 5) have no corresponding segments of the collar (Pl. I 7). Their posterior ends reach the termination of the peristome (its most deeply situated part leading to cytostome). The right marginal kinety is distinguished by bigger kinetosomes and by a rather distinct shifting aside of the complex of other kineties (Fig. 1, Pl. I 5, 6, 7). The peristomal kineties run obliquely to the axis of the peristomal cone and, as a result, the terminal segments of kineties pass upon the dorsal wall of the peristome. The number of kineties in the peristomal cone is in average 24 in the species *P. colpoidea,* and 20 in *P. minuta.* The location of the peristomal kineties is extremely compact and regular. Each kinety consists of a row of kinetosomes closely adhering to one another, producing sometimes an impression of UM. All the kineties, disposed densely in parallel to one another, are joined by transverse fibrils (Pl. I 5, 8, 9, II 15, 16, III 23 and V 38) and form a ciliated organelle of a higher rank which may be compared only to the peniculus of *Hymenostomata-Peniculina.*

Besides the ciliary equipment, a complex system of silver-impregnating fibers occurs in the peristome. The majority of them seem to be connected directly with kinetosomes. Their course is difficult to be disentangled, especially on the left peristome side, where the ventral and dorsal walls meet at an acute angle.

For convenience, the peristome fibers may be classified into several groups as to their position.

The first group consists of fibers with a semicircular course, lining the right peristome side and also partly the ventral and dorsal ones. The semicircular fibers begin at the level of the anterior end of the marginal peristome kinety; their number corresponds to the number of the kinetosomes of the marginal kinety and they seem to originate from them. (Fig. 1, and Pl. I 7, III 23, 26). This fiber group is seen distinctly, in the phase contrasting optics and even with the usual immersion lens, in living ciliates or in preserved in formalin. After silver impregnation (for methods see W o l s k a 1964)[1], they become distinct almost in their full course but the segments bent over the dorsal side are difficult to be observed. The aspect of those fibers and also their thickness is various, presumably depending on the intensity of impregnation. Sometimes they are stretched (Pl. I 5) in the other cases — folded (Pl. III 23). Each fiber, after having described a semicircle, terminates on the dorsal wall forming a swell. Often such swellings may be observed along the whole fiber which assumes then the appearance of a kinety (Pl. IV 33).

Another group of fibers — let us call them oblique — initiate at the left margin of the area of peristomal kineties, at their terminal segments (Pl. III 21 and IV 29) and at the terminal kinetosomes of the collar kineties. This group of fibers initiate at the same level as the semicircular fibers. The oblique fibers line the antero-dorsal wall of peristome (Pl. I 6 and IV 27). Neither in the living material nor after the formalin fixation those fibers were observed, they appear only after a strong silver impregnation. Just after having

[1] The silver impregnation method of Bielschowsky also was applied with very good results. All the other details of method were unchanged.

left the final segments of the peristomal kineties, these fibers turn sharply (Pl. I 9, II 11, 12, 13) to the dorsal peristome wall, bend slightly (Pl. I 6, III 28) and run obliquely towards its right side, there they turn under its right margin (Fig. 1 and Pl. V 35—37). Some oblique fibers, running from the terminal kinetosomes of the kineties of the shallow peristome part, diverge and spread in a fan-shaped pattern over the dorsal wall of peristome and approach the group of frontal cilia (Fig. 1 and Pl. IV 27). Fibers of this group are thinner than those of the group supporting the right margin. Swellings and foldings are observed on the oblique fibers (Pl. IV 27, 32 and V 35, 36).

To the third cathegory belongs a longitudinal fiber running along the line limiting the ends of the peristomal kineties and reaching the marginal kinety (Fig. 1). This fiber was never observed in living nor in formol fixed material but appeared very distinctly after silver impregnation when the ciliate was mounted in a convenient position. The longitudinal fiber appears in the form of a zigzag-line or, may be, it is twisted to form a helix. In some other cases it has a form of a more or less straightened line or is slightly folded (Pl. II 14, 16—18, III 19, 20, 24, IV 30, 31). In Pl. III 22 (silver-impregnated oblique section,) a folded segment of a longitudinal fiber near the peristomal kineties is seen. The longitudinal fiber is usually well marked between the deep part of the peristome and the first oblique fiber. Some images (e.g. Pl. II 16) seem to indicate that this fiber is prolonged to the anterior part of peristome. In some other cases (Pl. I 8, II 13, III 24—25), it can hardly be distinguished whether the twisted places of the longitudinal fibers, or segments of strongly impregnated oblique fibers, are seen.

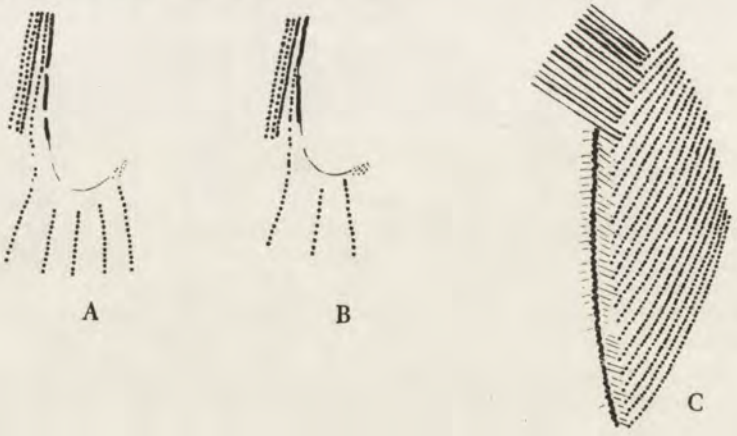

Fig. 2. Right peristome margin. A. *P. colpoidea* Fiorentini. B. *P. minuta* Hsiung. C. Longitudinal fiber and connective fibers in the genus *Paraisotricha* Fiorentini; in the superior part of diagram note the oblique fibers and at right — peristomal kineties, viewed from inside; semicircular fibers not revealed

The last group includes very thin and hardly observable fibers, defined in the present study as left and right connective fibers. They connect the longitudinal fiber with the ends of peristomal kineties on one side (left connective fibers), and with the ends of semicircular fibers on the other (right connective fibers). Pl. II 10 and III 19 represent — although not very distin-

ctly — the left connective fibers. The left and right connective fibers are visualized in the scheme (Fig. 2 C).

The preoral kinety on the right margin of the peristome in *P. colpoidea* is a non-continuous haplokinety composed of 2 or 3 segments, the third one being usually very short. Each segment of UM is built of very big kinetosomes so closely adherent to one another that after silver impregnation it assumes a feature of a quite uniform, thick black line (Fig. 2 A, Pl. I 1). In some few cases, the single kinetosomes — forming the UM — could be discerned. The position of UM left to the kinety 1 is distinct. In *P. minuta* the above pattern is less clear. The anterior segment of the kinety 1 is effaced and, possibly, fused with the anterior segment of UM. Probably the first segment of UM (out of two, seemingly occurring in *P. minuta*) is the segment of a kinety 1 with big and crowded kinetosomes (Fig. 2 B). The second UM segment (probably the only one really existing) is quite distinct and runs in parallel to the kinety 1. Whether it is a haplokinety or polykinety — no suggestion can be gathered from the silver impregnated material.

The free margin of the peristome, between UM and the collar, is — as a rule — not impregnated. Sometimes it is marked in *P. colpoidea* as a darker strand (Pl. II 1). The only case when the free margin of the peristome is impregnated is represented in Pl. IV 34. In *P. colpoidea* the free margin of the peristome is longer than in *P. minuta*, extending along the area from the kinety 1 to the kinety n−3 or n−4 (Fig. 2 A and Pl. I 1), while in *P. minuta* it extends from the kinety 1 to the kinety n−1 (Fig. 2 B, Pl. I 2).

The group of frontal cilia — co-operating with the cilia of peristome and of the collar — should be included to the oral ciliature, on account of their function.

The action of the peristomal and collar cilia can be observed easily in the living material. Those observations may be summarized as follows: 1. The cilia of the anterior part of peristome and of the collar, as well as the frontal cilia, are longer than the somatic ones; this fact has already been stated by the previous authors. 2. The work of the peristomal and collar cilia, as also that of the frontal ones, is independent from that of the somatic ciliature; it was often observed that the movement of the first ceases when the somatic cilia are active. 3. The cilia of the peristome and collar beat simultaneously — as a whole system; the frontal cilia work similarly.

In the majority of preparations of the genus *Paraisotricha,* the quasimotorium becomes impregnated. By using this term, the fact should be stressed that the feature and position of the organelle correspond to those disputable structures which were defined as motorium in other *Protozoa.* Quasimotorium is a strongly impregnable lobal mass with several processes, one of which being especially long and distinct (Pl. V 39—41). Quasimotorium lies in the right anterior part of the peristome near the "Konkrementenvakuole". Its short processes are directed towards the UM and the triad of short kineties (see W o l s k a 1964). The long process reaches as far as the anterior part of the left side of the peristomal cone.

Describing the "Konkrementenvakuole" in *Paraisotricha* sp., D o g i e l 1929 mentioned a spot of cytoplasm staining strongly with iron haematoxylin, situated in the left anterior corner of the peristome. This structure reminds the motorium as described in many other ciliates mostly by many American authors. The quasimotorium described in the present article occupies another

position than the mass mentioned by Dogiel since it lies in the right anterior part of the peristome.

No attempt is made in the present article to define the function of quasi-motorium — this task being beyond the scope of this investigation — and only the existence of this sort of organelle in the genus *Paraisotricha* is reported. The shape of this structure is characteristic and its position is constant; it is strongly impregnable with silver and cannot be looked upon as an artifact.

Rhizotricha beckeri (Hsiung) Wolska

The peristome in *Rhizotricha beckeri* is distinctly shifted towards the apical pole so that its dorsal edge either crosses the apex or passes closely beneath it (Fig. 3, Pl. VI 42, 45). The peristomal overture is ovoid with its sharp end turned towards the apical pole. On its right margin, in the anterior segment, lies a narrow stripe of preoral cilia homologous with frontal cilia in *Paraisotricha*. The posterior segment of the right margin of the peristome is occupied by UM, which passes over the ventral margin and terminates a short distance from the kinety n. This part of the peristome margin edges itself between the kinety 1 and kinety n deforming the ovoid outline of the buccal overture (Pl. VI 43). The UM is irregularly undulated and twisted in its terminal segment, when approaching the kinety n. When impregnated, it appears as a sharp, thick black line. After a delicate impregnation, it may be stated that UM is a polykinety composed of 2 or perhaps 3 kineties (Pl. VII 50, 52, 53). The thin ventral peristome margin and the left slightly decline outside, the strongest deflexion being marked in the left posterior corner. Over the ventral and the left peristome margins the long peristomal cilia protrude outside forming a sort of tongue-shaped structure. On the apex, the left anterior peristomal margin meets a strand of preoral cilia. The ciliature of the peristomal cavity in *Rhizotricha beckeri* resembles that in *Paraisotricha* but the peristomal kineties initiate in the margin (left ventral) of the peristome, and consequently, the collar is absent. The kineties of the left side are short; they line the anterior shallow part of the peristome (Fig. 3, Pl. VI 46). The kineties of the ventral wall lengthen gradually and run obliquely over the left ventral peristome wall. The terminal kineties — the most right — begin at a certain distance from the peristome margin. The right margin kinety — like in *Paraisotricha* — has bigger kinetosomes than the others, although this difference is not so strongly marked as in *Paraisotricha*.

The whole ciliature of the peristome is in *Rhizotricha* finer than in *Paraisotricha*, the kinetosomes are smaller and more densely located. A short segment of the ventral peristome margin neither supported by the peristomal kineties to the left of the kinety n, nor by UM to the right of it, is for that reason, scarcely visible. Left of the kinety n, the margin is slightly depressed, right of it — it falls abruptly between the anterior end of the kinety n and the termination of UM.

The peristome fibers are in *Rhizotricha* thinner and their silver impregnation is weeker than in *Paraisotricha*. Determining their mutual relations, and their relation to other elements, is more complicated. The semicircular and oblique fibers, as well as the longitudinal fiber with a course similar to that in *Paraisotricha*, may also be distinguished (Fig. 3, Pl. VI 45, 47). The semicircular fibers initiate at the kinetosomes of the marginal peristome kinety,

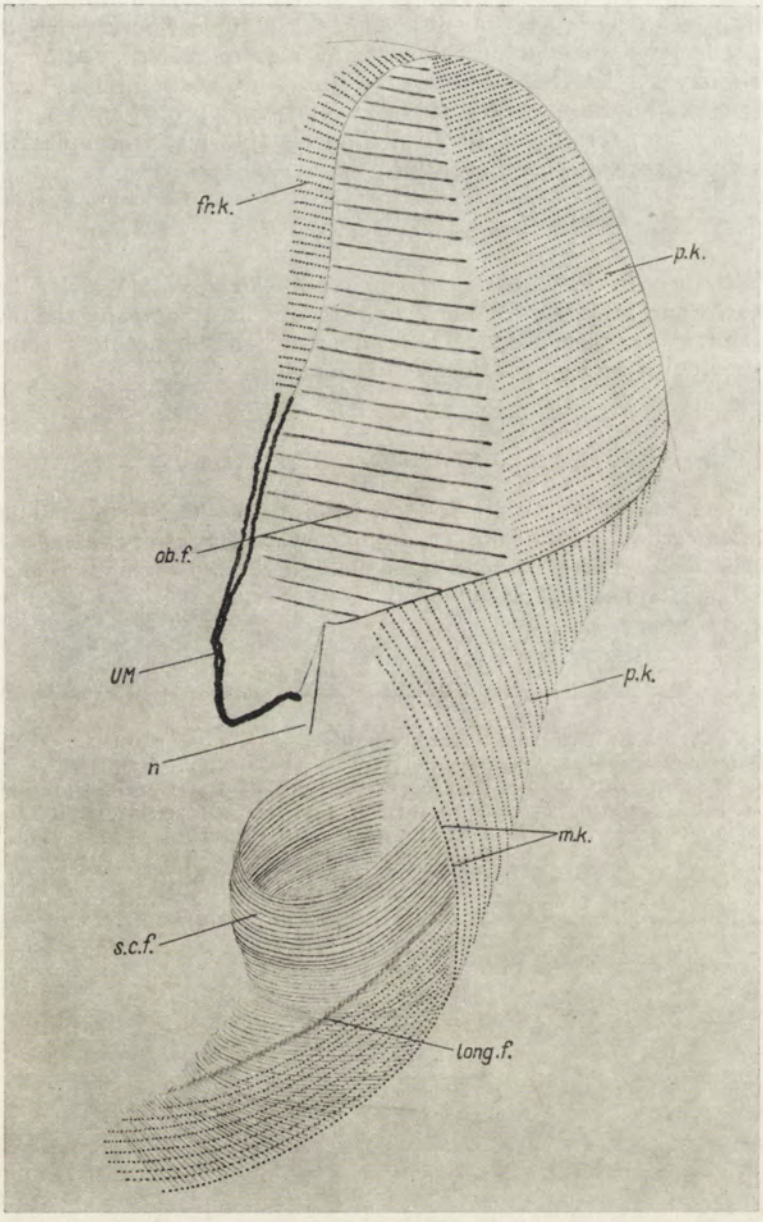

Fig. 3. Peristome of *Rhizotricha beckeri* (Hsiung);
fr.k. — frontal kineties, p.k. — peristomal kineties, m.k. — marginal kineties, long. f. — longitudinal fiber, s.c.f. — semicircular fibers, n — kinety n, UM — undulating membrane, ob.f. — oblique fibers

the oblique ones seem to be grouped, several together, since their number on the dorsal peristome wall (Fig. 3) is lower than the number of kineties lining this part of peristome. As it seems, the oblique fibers reach the right margin like in *Paraisotricha* but this was never quite evident. Neither the connective fibers were detected, however the similitude of the other fiber systems in both species allows to presume their existence very probable.

Quasimotorium, forming a fusiform impregnable mass, occupies the same position as in *Paraisotricha* Fiorentini.

Summary

Oral ciliature in *Paraisotricha* Fior. and *Rhizotricha* Wolska is highly differentiated and integrated. Four systems of fibrils accompany the oral kineties. Peristomal kineties produce a complex organelle, not less complicated than the peniculus in *Hymenostomata—Peniculina.*

STRESZCZENIE

Orzęsienie gębowe u *Paraistoricha* Fior. i *Rhizotricha* Wolska jest silnie zróżnicowane i zintegrowane. Kinetom gębowym towarzyszą cztery układy włókien. Kinety peristomalne tworzą złożone organellum, nie mniej skomplikowane niż peniculus u *Hymenostomata—Peniculina.*

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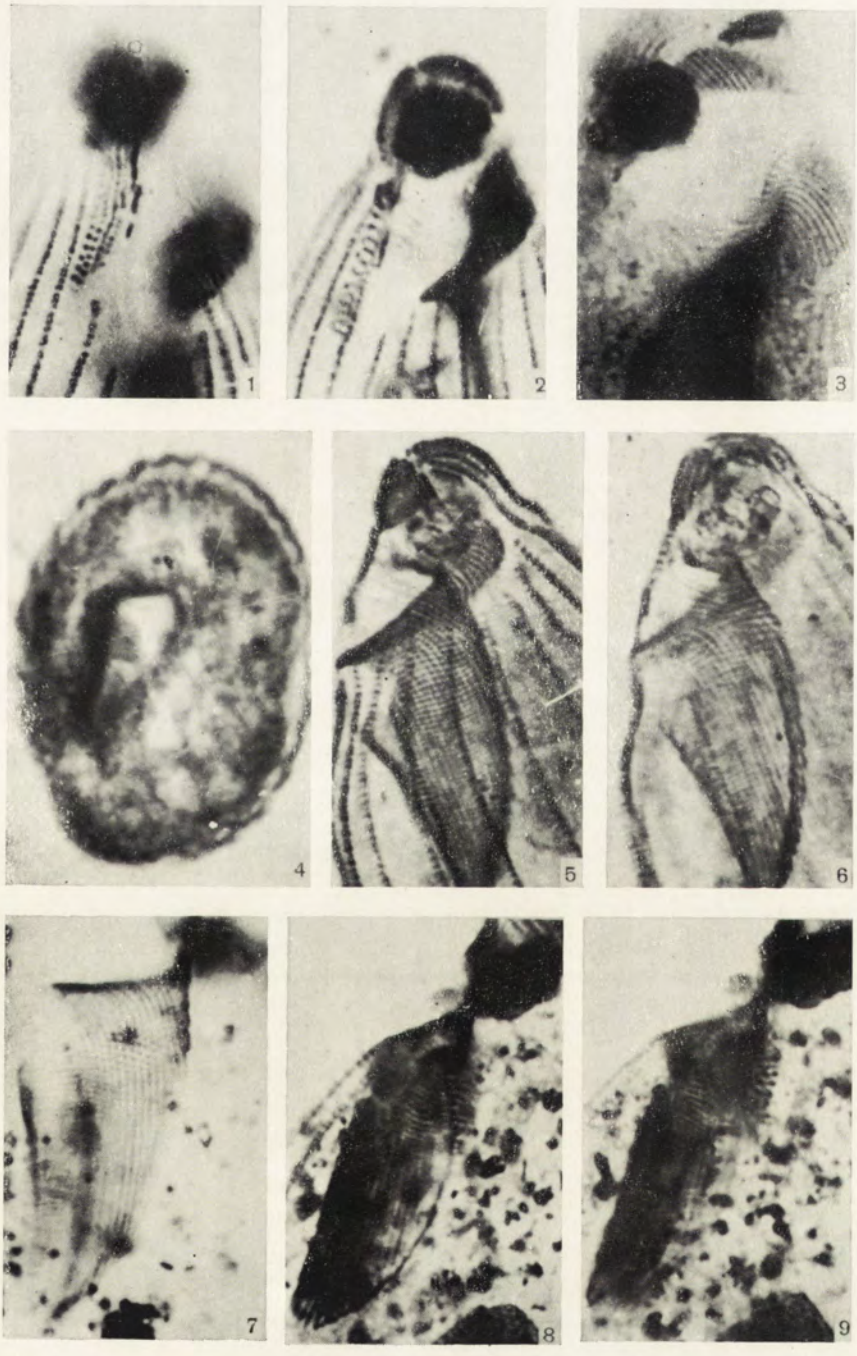

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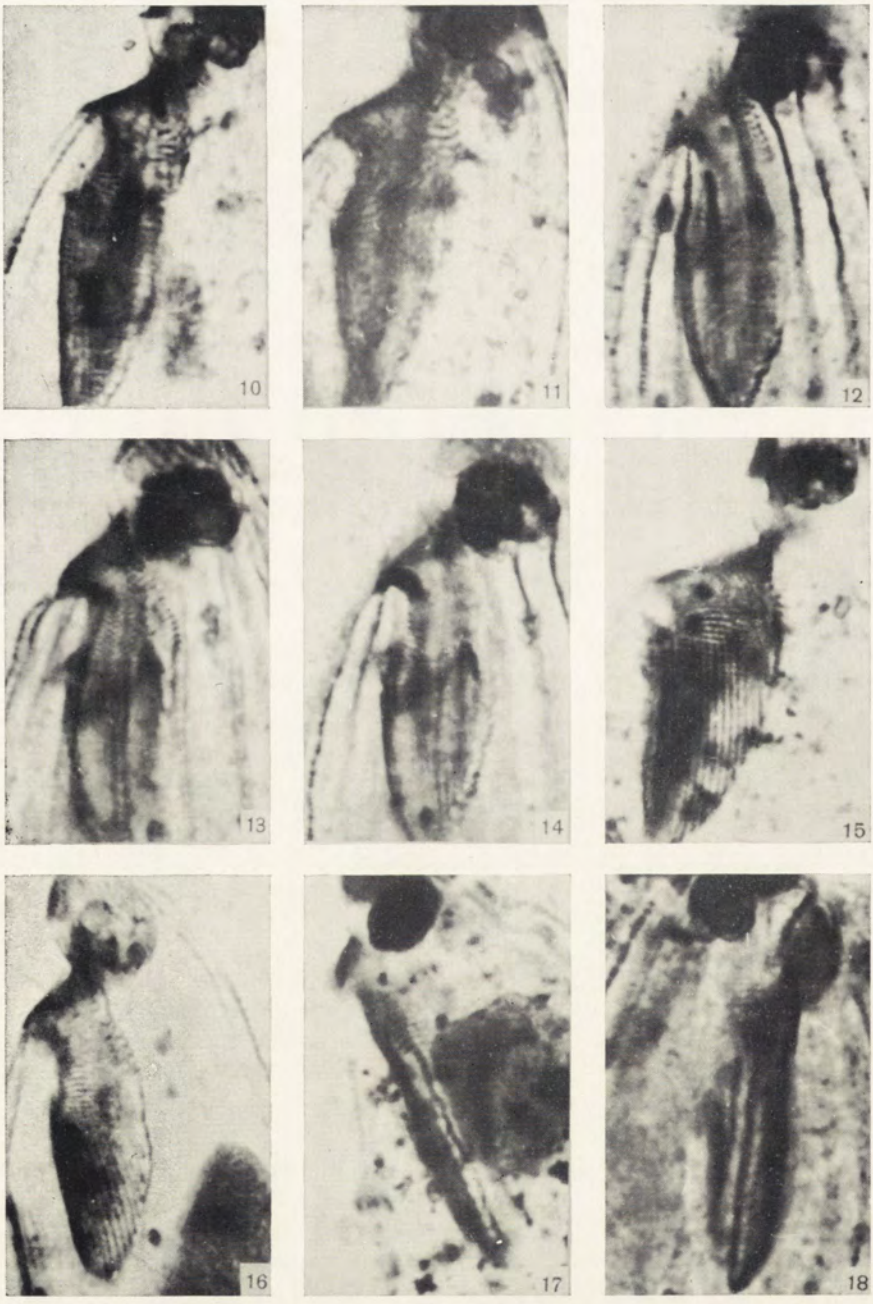

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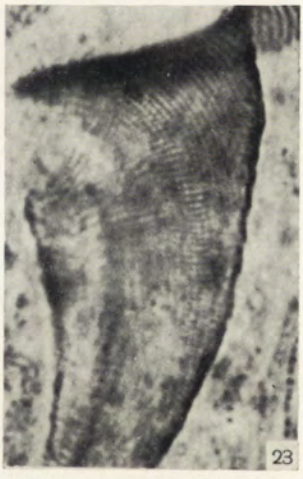
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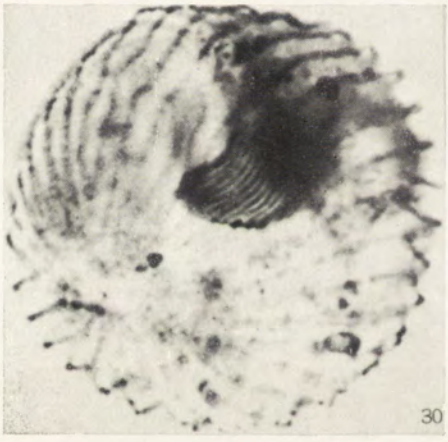
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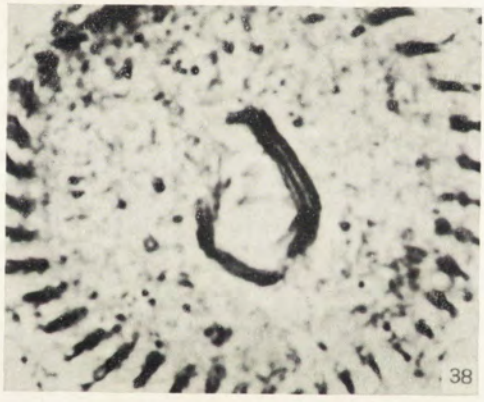
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EXPLANATION OF PLATES I—VII

1: *Paraisotricha colpoidea* Fior., the left peristome margin, 3 segments of UM, outline of the free margin (×2000).
2: *Paraisotricha minuta* Hsiung, the right peristome margin, 2 segments of UM (× 2000).
3: *P. colpoidea*, anterior part of peristome, collar (×2200).
4: *P. colpoidea*, oblique section below the "Konkrementvakuole", outline of the peristome lumen (×2200).
5: *P. minuta*, peristome in surface view, on the left peristomal kineties are seen (×2200).
6: *P. minuta*, optic section of the peristome (×2200).
7: *P. minuta*, peristome seen from the left side in optic section (×2200).
8, 9: *P. minuta*, peristome seen from the left side in optic section on two different levels (×2000).
10: *P. minuta*, peristome seen from the left side in optic section (×2000).
11: *P. minuta*, peristome from the left side, optic section (×2000).
12—14: *P. minuta*, peristome seen from the left side in optic section at 3 different levels (×2200).
15: *P. minuta*, optic section of the peristome (left side), cross fibers connecting the peristomal kineties are seen (×2000).
16: *P. minuta*, optic section, left side view, longitudinal fiber in peristome (×2000).
17: *P. minuta*, optic section, left dorsal view, longitudinal fiber, in the anterior part oblique fibers turned to the dorsal side (×2000).
18: *P. minuta*, optic section of the peristome, right ventral view, logitudinal fiber is seen (×2020).
19: *P. minuta*, left dorsal view, ends of peristomal kineties and longitudinal fiber are seen (×2000).
20: *P. minuta*, optic section, right ventral view, longitudinal fiber is seen (×2000).
21: *P. minuta*, optic section, right ventral view, oblique fibers are seen (×2200).
22: *P. minuta*, silver impregnation, oblique section, peristome kineties and longitudinal fiber are seen (×2200).
23: *P. colpoidea*, peristome, cross fibers connecting the peristome kineties are seen (×2200).
24: *P. minuta*, optic section, left side view, longitudinal fiber is partly distended (×2200).
25: *P. minuta*, right ventral view, optic section (×2200).
26: *P. minuta*, optic section of peristome, right ventral view, semicircular fibers (×2000).
27: *P. minuta*, optic section of anterior part of peristome, ventral view, oblique fibers are seen (×2000).
28: *P. minuta*, optic section, peristome seen from the left dorsal side, bent oblique fibers (×2000).
29: *P. minuta*, optic section of peristome, right side view, oblique fibers running from the peristome kineties are seen (×2000).
30: *P. colpoidea*, viewed from the posterior pole, peristome kineties and partly longitudinal fiber are seen (×2000).
31: *P. minuta*, as above, longitudinal fiber is twisted (×2000).
32: *P. minuta*, optic section, oblique fibers are folded approaching the right peristome margin (×2000).
33: *P. minuta*, optic section, ventral view, semicircular fibers with swellings (×2200).
34: *P. colpoidea*, the right posterior margin of peristome is impregnated (×2000).
35, 35a, 36: *P. minuta*, optic section of peristome, right ventral view at two different planes; 35 — oblique folded fibers, 36 — fibers beneath the right margin, in the posterior part of peristome connecting fibers (×2000).
37: *P. minuta*, as above, oblique fibers turn under the right margin (×2000).
38: *P. colpoidea*, cross section beneath the "Konkrementvacuole", silver impregnation, cross fibers of the peristome kineties are seen (×2200).
39: *P. minuta*, optic section of the anterior part of peristome, near the right margin quasimotorium (×2000).

40: *P. colpoidea*, as above (×2200).
41: *P. minuta*, as above (×2200).
42: *Rhizotricha beckeri* (Hsiung), anterior body end, position of peristome, left side view (×2000).
43: *R. beckeri*, anterior body end, ventral side view, double UM (×2000).
44: *R. beckeri*, anterior body end, right dorsal view, UM is folded (×2000).
45: *R. beckeri*, optic section of peristome, left side view (×2000).
46, 47: *R. beckeri*, the peristome; 46 — double UM in the anterior segment, 47 — peristome in optic section, fibers on the dorsal wall of peristome (×2000).
48, 49: *R. beckeri*, optic section of peristome in two different planes (×2000).
50: *R. beckeri*, fibers on the dorsal wall of peristome, quasimotorium (×2000).
51: *R. beckeri*, optic section of peristome, quasimotorium (×2200).
52: *R. beckeri*, viewed from the apical pole, double UM (×2000).
53: *R. beckeri*, optic section from the dorsal side, UM is double or possibly triple (×2000).

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О развитии некоторых пироплазмид в организме позвоночных хозяев

On the development of some piroplasmidae in the vertebrate hosts

В течение четырех лет (1960—1963 гг.) мы проводили исследования, целью которых была расшифровка некоторых неясных сторон жизненных циклов пироплазмид.

Эксперименты проводились с *Piroplasma bigeminum*, *Babesiella bovis* и с нутталлиями краснохвостой песчанки (*Meriones erythrourus*). При проведении работы мы стремились выяснить, на какой день после подсадки клещи передают инвазию и что происходит с пироплазмидами в организме теплокровного хозяина от момента инокуляции клещем до проявления клинической картины и обнаружения паразитов в мазках периферической крови методом микроскопии. Исчезают ли паразиты из кровяного русла позвоночного хозяина после введения клещем или в течение всего инкубационного периода присутствуют в нем, и могут ли клещи-переносчики инвазироваться, питаясь на зараженных животных в инкубационный период.

Имеющиеся в настоящее время сведения о развитии пироплазмид в позвоночном хозяине в инкубационный период крайне бедны. Относительно хорошо изучены для некоторых пироплазмид лишь сроки передачи инвазии клещем позвоночному хозяину. Так Regendanz und Reichenow 1933 сообщают, что *Babesia canis* передается клещем *Dermacentor reticulatus* на 3—4-й день после подсадки. Наблюдения Reusse 1954 подтверждают данные Regendanz und Reichenow; он показал, что *B. canis* и *B. vogeli* инокулируются клещем *Hemaphisalis leachi* на 4-й день. Абрамов, Цапрун, Степанова и Лебедев 1952 считают, что заражение лошадей *Nuttallia* половозрелыми клещами *Dermacentor marginatus* наступает через 3—4 дня после их прикрепления. Feldman-Muhsam 1958 был получен перенос нутталлий песчанки клещем *Rhipicephalus secundus* на 4-й день и в одном случае через 24 часа после подсадки. Hoyte 1961 получил передачу *Babesia bigeminum* клещем *Boophilus microplus* лишь между 8 и 9 днем после прикрепления личинок.

Данных же о наличии или отсутствии пироплазмид в крови позвоночного хозяина в течение всего инкубационного периода и их способности инвазировать в это время клещей пока не имеется. Неясно также в эритроцитах ли размножаются пироплазмиды в инкубационный период или размножение их необязательно связано с внутриэритроцитарной локализацией.

Отсутствие этих сведений и побудило нас к постановке настоящих исследований.

Считаю своим долгом выразить большую благодарность проф. Е. М. Хейсину за ценные методические советы.

Материал и методика

Во всех опытах участвовали животные, стерильные в отношении кровепаразитарных заболеваний. Крупный рогатый скот вывозился из благополучной по кровепаразитарным заболеваниям местности, песчанки выращивались в лаборатории.

Клещи-переносчики, использовавшиеся для заражения, культивировались в лаборатории. Личинки *Boophilus calcaratus* получены от самок, кормившихся на крупном рогатом скоте, остро переболевающем пироплазмозом (возбудитель — *P. bigeminum*). *Ixodes ricinus* — переносчик бабезиеллеза (возбудитель — *B. bovis*) и *Hyalomma anatolicum* — переносчик нутталлиоза песчанок (возбудитель — *N. tadzhikistanica*) кормились в воспринимающих инвазию фазах на больных спленэктомированных животных соответствующих видов с очень сильной паразитарной реакцией. Такая подготовка клещей производилась с целью получения четких результатов в опытах при заражении через них позвоночных хозяев.

Для выяснения сроков передачи инвазии клещами на подопытных животных подсаживались зараженные клещи-переносчики (от сотни до нескольких тысяч) и затем через различное время убивались акарицидом (гексахлораном).

Обнаружение пироплазмид в крови позвоночного хозяина в инкубационный период проводилось путем переливания в различные сроки инкубационного периода крови от животного-донора, зараженного через клещей-переносчиков, стерильным животным-реципиентам.

С целью выяснения локализации нутталлий в позвоночном хозяине у зараженных через клещей песчанок в различные периода инвазии исследовались мазки и отпечатки из внутренних органов и тканей. Препараты окрашивались по Романовскому.

У подопытных животных ежедневно исследовались мазки периферической крови и измерялась температура тела. В некоторых случаях для получения более четкой паразитарной реакции животные спленэктомировались.

Сроки передачи инвазии

Piroplasma bigeminum (Smith et Kilborne, 1893)

Опыты по выяснению сроков передачи инвазии клещей *Boophilus calcaratus* проводились в Ленинградском научно-исследовательском ветеринарном институте на местных животных, безусловно стерильных в отношении *Piroplasma bigeminum*. Клещи-переносчики, зараженные *P. bigeminum*, были завезены из Таджикистана.

В эксперименте использовано 13 бычков. На каждого бычка подсаживалось от 9 до 10 тысяч личинок *B. calcaratus*, полученных от разных самок. После подсадки на бычков личинки *B. calcaratus* были убиты гексахлораном на двух бычках (№№ 19, 20) через 2 дня, на трех (№№ 21, 22, 23) через 4 дня и затем через 6 (на бычках №№ 24, 25), 7 (на бычках №№ 26, 27), 9 (на бычках №№ 28, 29) и через 10 дней (на бычках №№ 30, 31). Наблюдения за подопытными животными, проводившиеся в течение 30 дней показали, что заразились лишь те животные, на которых клещи кормились 9—10 дней (Таблица 1). Бычки же, на которых клещи были убиты через 2, 4, 6 и 7 дней после подсадки, не проявили никаких признаков заражения *P. bigeminum*.

Таблица 1

Сроки передачи *P. bigeminum* клещами *B. calcaratus* (количество подсаженных личинок клещей: 9000 10 000)

Time of transmission of *P. bigeminum* by the tick *B. calcaratus* (number of the tick larve used: 9000—10 000)

№ подопытных животных Animal Nr.	На какой день после подсадки клещи убиты акарицидом Day of killing the ticks	На какой день после подсадки клещей обнаружены паразиты Day of detection of parasite
19 20	2	—
21* 22* 23	4	—
24* 25	6	—
26 27	7	—
28 29	9	16 18
30 31	10	16 20

* спленэктомированное животное — splenectomised animal.

Опыты по переливанию крови в инкубационный период, проведенные нами в Таджикистане (Крылов 1962) на стерильных в отношении *P. bigeminum* животных, вывезенных из высокогорных районов, показали несколько иную картину.

В одном опыте кровь переливалась от бычка-донора, зараженного через клещей, бычкам-реципиентам на 1, 2, 6 и 9-е сутки после подсадки клещей. Ни одно животное-реципиент не заразилось пироплазмами. У бычка-донора первые паразиты были обнаружены на 16-й день после подсадки клещей.

В другом опыте, где на бычка-донора было подсажено свыше 20 000 личинок *B. calcaratus* и кровь переливалась на 2, 4, 6, 8, 11 и 14-е сутки после подсадки

клещей, заразились все бычки-реципиенты, которым была введена кровь от бычка-донора, начиная с 4-го дня после подсадки личинок. Не заразился лишь один бычок-реципиент, получивший иньекцию крови, взятой на 2-е сутки после подсадки клещей.

Таким образом, проведенные исследования показывают, что введение *P. bigeminum* в кровь позвоночного хозяина клещами *B. calcaratus* производится нимфами на 9-й день после подсадки личинок. Эти сроки совпадают с данными Ноуте 1961. Однако, в одном опыте передача инвазии была осуществлена личинками на 4-й день после их подсадки. Столь раннюю передачу инвазии мы считаем возможным объяснить за счет подсадки большого количества личинок *B. calcaratus*. Это тем более вероятно, что опытами Абрамова, Цапруна, Степановой и Лебедева 1952 была показана определенная зависимость между количеством подсаженных личинок *Rhipicephalus bursa* и передачей ими *Babesiella bovis*. Авторами установлено, что при подсадке личинок в количестве менее тысячи заражения не наступает.

Видимо, период, необходимый для развития инвазионных форм пироплазмид, может варьировать. В опытах Feldman-Muhsam 1958 по выяснению времени, необходимого для развития заражающих форм нутталлий песчанок в клеще *Rhipicephalus secundus* было показано, что этот период может колебаться от 24 часов до 4 дней.

Babesiella bovis (Babes, 1888)

Первоначально были поставлены эксперименты по обнаружению бабезиелл в крови позвоночного хозяина в инкубационный период (Гусев и Крылов 1963). С этой целью на бычка-донора было подсажено 100 нимф *Ixodes ricinus*, зараженных *Babesiella bovis*, и от него переливалась кровь (по 250—300 мл) стерильным спленэктомированным бычкам-реципиентам на 2, 4, 6 и 8-е сутки после подсадки клещей. Было установлено, что *B. bovis* обнаруживается в крови биопробой на 4-й день после подсадки клещей, при длине инкубационного периода 9 дней.

Позже мы по такой же схеме поставили опыт по обнаружению *B. bovis* в крови бычка-донора между 2 и 4 днем инкубации. Оказалось, что даже через 52 часа после подсадки клещей бабезиеллы появляются в крови позвоночного хозяина (Таблица 2).

Затем были проведены исследования на 4-х животных по выяснению сроков передачи *B. bovis* клещами *I. ricinus*. На каждого из 4-х стерильных спленэктомированных бычков было подсажено по 100 нимф *I. ricinus*, зараженных *B. bovis*. Клещи были убиты гексахлораном на одном бычке через 24 часа, на втором через 48, на третьем через 72 и на четвертом через 96 часов после подсадки. Бабезиеллезом заразились животные, на которых клещи были убиты через 48, 72 и 96 часов после подсадки. Животное, на котором клещи кормились лишь сутки, осталось незараженным (Таблица 3).

Опыты с подсадкой и последующим уничтожением в различные сроки клещей показали, что *I. ricinus* передает инвазию через 48 часов после подсадки на позвоночного хозяина.

В опытах с переливанием крови в инкубационный период от бычка-донора, зараженного *B. bovis* через клещей, стерильным бычкам-реципиентам установлено, что бабезиеллы могут быть обнаружены в кровяном русле позвоночного хозяина в любой период инкубации, начиная с 52 часов после подсадки зара-

Таблица 2

Опыт по переливанию крови в инкубационный период от бычка-донора зараженного *B. bovis* через клещей, стерильным бычкам-реципиентам

Experiments on the blood transfusion from a bull-donor, infected with *B. bovis* by the ticks, to a bull-recipient, during the incubation period

№ и назначение животных Animal's destination and Nr.		Через сколько часов после подсадки клещей перелита кровь Hour of transfusion	На какой день после заражения обнаружены бабезиеллы Day of detection of parasite
бычок-донор bull-donor	32		9
бычок-реципиент (перелита кровь от бычка № 32) bull-recipient (transfusion from the bull Nr. 32)	33	48	не обнаружено non detected
	34	96	7
	35	144	5
	36	192	4
бычок-донор bull-donor	37		8
бычок-реципиент (перелита кровь от бычка № 37) bull-recipient (transfusion from the bull Nr. 37)	38	52	8

Таблица 3

Сроки передачи *B. bovis* клещами *I. ricinus* (количество подсаженных нимф клещей — 100)

Time of transmission of *B. bovis* by the tick *I. ricinus* (number of the tick nymphae used — 100)

№ подопытных животных Animal Nr.	Через сколько часов после подсадки клещи убиты акарицидом Hour of killing the ticks	На какой день после подсадки клещей обнаружены паразиты Day of detection of parasite
39	24	—
40	48	7
41	72	8
42	96	9

женных клещей. Мы не видим какого-либо существенного разрыва во времени между инокуляцией клещем *B. bovis* и последующим обнаружением бабезиелл в крови позвоночного хозяина биопробой.

Также, как и в опытах с *P. bigeminum*, *B. bovis* не исчезает из кровяного русла позвоночного хозяина, а весь период от момента инокуляции клещем до проявления клинической картины обнаруживаются в нем.

Nuttallia tadzhikistanica (Krilov et Zanina, 1962)

Принципиально та же схема как и в опытах с *P. bigeminum* и *B. bovis* была использована при работе с *N. tadzhikistanica* на песчанках.

Предыдущими исследованиями (Крылов 1963) установлено, что нутталлий краснохвостой песчанки переносит клещ *Hyalomma anatolicum*. Передача инвазии идет трансфазально от личинки к нимфе, трансовариальная передача отсутствует.

Все песчанки, участвовавшие в опытах, родились и выросли в лаборатории. Содержались подопытные животные в условиях, исключающих возможность их спонтанного заражения нутталлиями.

В опытах по переливанию крови в различные сроки инкубационного периода было использовано 9 животных. На песчанку-донора № 46 было подсажено 50 нимф *H. anatolicum*, зараженных нутталлиями. От песчанки-донора на 2, 4, 6, 8, 10, 12, 14 и 16-е сутки после подсадки клещей из кончика хвоста бралось 5—6 капель крови, смешивалось с физиологическим раствором и вводилось подкожно соответственно песчанкам №№ 47, 48, 49, 50, 51, 52, 53, 54.

Ежедневные исследования мазков крови у песчанок-реципиентов показали, что нутталлиями заразились все животные, которым была введена кровь, взятая от песчанки-донора, начиная с 4-го дня и позже после подсадки клещей-переносчиков. Незараженной осталась песчанка, получившая инъекцию крови, взятой у песчанки-донора на 2-й день после подсадки клещей (Таблица 4). Следует отметить, что паразитарная реакция у песчанок, инвазированных кровью, взятой в первой половине инкубационного периода, протекала значительно слабее, чем у песчанок, зараженных кровью, полученной в конце инкубационного периода. Так у песчанки, получившей инъекцию крови, взятой на 4-й день инкубационного периода от песчанки-донора при длине инкубационного периода 16 дней, был обнаружен лишь один паразит за 2 месяца наблюдений, в то время как у песчанки, получившей инъекцию крови, взятой на 14-й день инкубации, паразиты встречались весь период наблюдения (2 месяца).

Для того, чтобы установить имеется ли разрыв во времени между инокуляцией нутталлий клещем и появлением их в кровяном русле позвоночного хозяина, необходимо было решить вопрос, на какой день после подсадки клещи *H. anatolicum* передают нутталлий песчанке.

С этой целью на каждую из 12 стерильных песчанок было подсажено по 50 зараженных нутталлиями нимф *H. anatolicum*. Через 1, 2, 3, 4 и 5 суток после подсадки клещи убивались гексахлораном. Заразились нутталлиями лишь те 4 песчанки, на которых клещи находились 4 и 5 дней. У одной песчанки паразиты обнаружены на 14-й день, у двух на 18-й день и у одной на 22-й день после подсадки клещей. Песчанки, на которых клещи питались 1, 2 и 3 дня, остались не инвазированными нутталлиями (Таблица 5).

Сроки появления нутталлий в крови позвоночного хозяина (4-й день после подсадки клещей) и передачи инвазии клещами (также 4-й день) совпадают,

Таблица 4

Опыт по переливанию крови в инкубационный период от песчанки-донора, зараженной *N. tadzhikistanica* через клещей, стерильным песчанкам-реципиентам

Experiments on the blood transfusion from a *Meriones*-donor, infected with *N. tadzhikistanica* by the ticks, to a *Meriones*-recipient, during the incubation period

№ и назначение животных Animal's destination and Nr.		На какой день после подсадки клещей перелита кровь Day of transfusion	На какой день после заражения в мазках крови обнаружены паразиты Day of detection of parasite
Песчанка-донор *Meriones*-donor	46		16
	47	2	—
	48	4	26
	49	6	23
Песчанка-реципиент	50	8	22
Meriones-recipient	51	10	23
	52	12	23
	53	14	23
	54	16	23

Таблица 5

Сроки передачи *N. tadzhikistanica* клещами *H. anatolicum*
(— отрицательно, + обнаружены нутталлии)

Time of transmission of *N. tadzhikistanica* by the tick *H. anatolicum* (— nuttalliae non detected, + detected)

№№ подопытных песчанок Meriones Nr.	Через сколько дней после подсадки клещи убиты акарицидом Day of killing the ticks	Дни наблюдений и результаты исследовании мазков крови после начала опыта Days from the beginning of experiment and results of the investigation of blood smears							
		5	10	14	18	22	24	28	32
55, 56	1	—	—	—	—	—	—	—	—
57, 58, 59	2	—	—	—	—	—	—	—	—
60, 61, 62	3	—	—	—	—	—	—	—	—
63	4	—	—	—	+	+	+	+	+
64		—	—	—	—	+	+	+	+
65	5	—	—	+	+	+	+	+	+
66		—	—	—	+	+	+	+	+

следовательно, инвазионные формы нутталлий, вводимые клещем позвоночному хозяину, проникают в кровяное русло последнего и, как мы видели в опытах с переливанием крови, весь инкубационный период присутствуют в нем.

Изучение преэритроцитарных форм паразита

Полученные данные ставят под сомнение возможность существования внутриклеточных преэритроцитарных форм у изучаемых пироплазмид.

Решение вопроса о наличии или отсутствии пироплазмид в инкубационный период в кровяном русле позвоночного хозяина дает некоторое представление о судьбе паразитов в этот период развития, однако, для того, чтобы окончательно судить о том, размножаются или нет пироплазмиды где-нибудь в других органах и тканях кроме крови, этих экспериментов явно недостаточно. Поэтому мы предприняли ряд опытов с применением других методов исследования.

Результаты экспериментов, проведенных в этом направлении с *P. bigeminum* подробно изложены в предыдущей нашей работе (Krylov 1964). Нам удалось обнаружить в капиллярах внутренних органов внеэритроцитарные формы множественного деления *P. bigeminum*, лежащие всегда внеклеточно в плазме крови.

Более подробные исследования были выполнены с *N. tadzhikistanica* на песчанках. Для изучения форм *N. tadzhikistanica*, развивающихся в позвоночном хозяине, было поставлено 4 опыта.

В первом опыте проводились поиски форм развития нутталлий в коже. Шести песчанкам были введены внутрикожно, на скарифицированную поверхность кожи и подкожно растертые с физиологическим раствором зараженные нутталлиями нимфы. Из них трем песчанкам (№№ 67, 68, 69) введены растертые голодные нимфы и следующим трем (№№ 70, 71, 72) — питавшиеся 96 часов на стерильных животных. После заражения через 1, 2 и 3 суток от всех подопытных песчанок бралась для исследования биопсия ткани в местах введения растертых нимф *H. anatolicum*. Ни в одном случае не удалось обнаружить каких-либо форм развития нутталлий. Заразились нутталлиями песчанки, которым были введены растертые нимфы *H. anatolicum*, питавшиеся 96 часов, заражение голодными нимфами не имело успеха. Проведенные исследования показали, что в нимфах, питавшихся 4 дня и использованных для заражения песчанок, были инвазионные формы нутталлий, но в коже и подкожной соединительной ткани они, видимо, не размножаются, а быстро поступают в ток крови.

Во втором опыте, поставленном на 18 песчанках, проводились поиски форм развития нутталлий во внутренних органах и тканях.

С этой целью 9 из 18 песчанок были спленэктомированы для обострения инвазии и 9 оставлены интактными. На всех песчанок были подсажены нимфы *H. anatolicum*, зараженные нутталлиями. Затем на 4, 6, 9, 12, 14, 16, 18, 20 и 25-й день после подсадки нимф забивалось по паре песчанок (одна спленэктомированная, одна интактная), от них исследовались мазки крови и отпечатки печени, селезенки, почек, легких, головного и спинного мозга.

Впервые мелкие (1.9—2.5 μ) одноклеточные амебовидные формы нутталлий, свободно лежащие в просвете капилляра, были обнаружены на 6-й день в почках у одной и на 9-й день в сердечной мышце у другой интактных песчанок (Рис. 1 A—E). Самые тщательные исследования периферической крови у песчанок в этот период не позволили выявить эритроцитарных форм нутталлий.

Обычно в крови методом микроскопии паразиты обнаруживаются лишь на 14—22-й день после подсадки клещей (Крылов 1963). Можно думать, что это были формы, предшествующие эритроцитарным, однако, судить о том, размножаются они или нет, невозможно, так как во всех случаях они были одноклеточными, без каких-либо признаков деления ядра. Аналогичные амебовидные

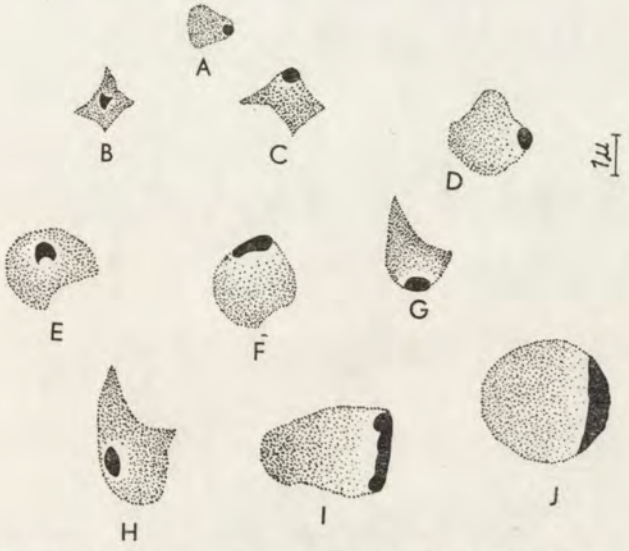

Рис. 1. Внеэритроцитарные формы *N. tadzhikistanica*, обнаруженные в инкубационный период
Fig. 1. Exoerythrocytal forms of *N. tadzhikistanica* detected in the period of incubation

стадии нутталлий (Рис. 1 F—J) были найдены в капиллярах легких на 20-й день после подсадки клещей, причем в крови также не удалось найти эритроцитарных форм паразитов.

Этими небольшими находками исчерпываются наши наблюдения над формами, предшествующими, по-видимому, эритроцитарным стадиям развития паразита.

Следует указать, что пироплазм вне эритроцитов видели Драчинский 1903, Kinoshita 1907, Yakimoff und Rastegaeff 1931, Delpy 1946, Cerruti 1962 и другие исследователи. Однако было неясно, выпавшие ли это из эритроцитов формы или переселяющиеся из эритроцита в эритроцит паразиты, размножаются ли они в плазме крови или размножение их связано только с внутриэритроцитарной локализацией.

В наших опытах у 3-х из 7 песчанок наряду с эритроцитарными формами были найдены паразиты и вне эритроцитов. У одной песчанки на 20-й день и у двух на 25-й день после подсадки клещей.

Внеэритроцитарные формы обнаружены в капиллярах легких, почек, печени, сердечной мышцы, селезенки и головного мозга. Во всех случаях паразиты лежали вне клеток, свободно в плазме крови. Размеры внеэритроцитарных форм варьировали в пределах от 1.25 до 6.25μ в ширину и от 1.25 до 7.5μ в длину. Причем между мелкими одноядерными амебовидными формами (Рис.

2 A—F) и крупными многоядерными паразитами (Рис. 3 P—S) встречаются все переходные (Рис. 2 G—K и 3 E—O), что указывает на превращение мелких одноядерных форм в крупные многоядерные. Деление многоядерных плазмодиев идет асинхронно, с одной из сторон от него отпачковываются одно- (Рис. 3 R), реже двуядерные (Рис. 3 N—O) паразиты. Полиморфизм обнаруженных паразитов говорит о их большой амебоидной подвижности.

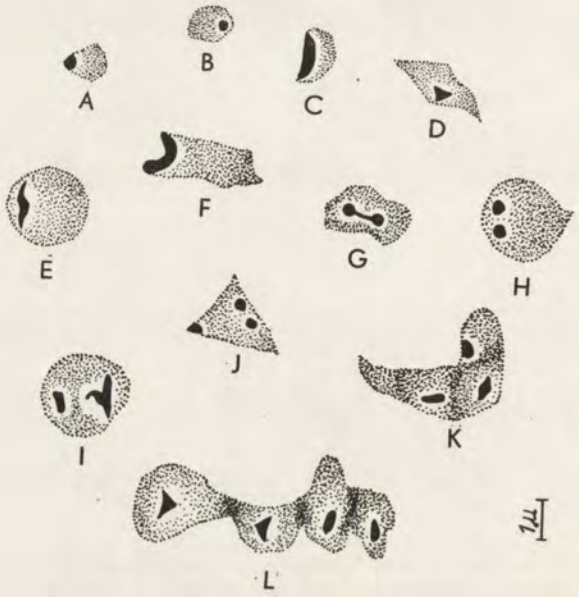

Рис. 2. Внеэритроцитарные формы *N. tadzhikistanica*, обнаруженные в период проявления клинических признаков заболевания
Fig. 2. Exoerythrocytal forms of *N. tadzhikistanica* detected in the period of manifestation of the clinical symptoms of disease

Таким образом, у *N. tadzhikistanica*, как и у *P. bigeminum*, наряду с типичными эритроцитарными формами имеются формы внеэритроцитарного размножения. Можно было думать, что внеэритроцитарные формы представляют собой обыкновенные эритроцитарные стадии паразитов, выпавших из эритроцитов или переселяющихся из эритроцита в эритроцит. Такие формы действительно можно наблюдать, особенно часто в период сильного разрушения эритроцитов, однако, обнаруженные нами внеэритроцитарные стадии отличаются от эритроцитарных размерами, формой и множественным делением.

Для того, чтобы выяснить продолжительность нахождения внеэритроцитарных форм паразитов в позвоночном хозяине, мы заразили нутталлиями через клещей 8 песчанок и затем через различные сроки (на 5, 10, 15, 25 и 30-й день) спленэктомировали их для обострения инвазии. Забивались эти песчанки также в различные сроки патентного периода (на 10, 15, 20, 25, 30, 40 и 50-й день). От забитых животных исследовались те же органы, что и в предыдущем опыте. У пяти из 8 подопытных песчанок были найдены внеэритроцитарные формы нутталлий, свободно лежащие в плазме крови капилляров печени, почек, легких,

сердечной мышцы, головного и спинного мозга. Внеэритроцитарные формы у одной из песчанок обнаружены даже на 50-й день патентного периода. Расположение, форма и размеры обнаруженных внеэритроцитарных паразитов ничем не отличались от аналогичных форм, обнаруженных в инкубационный период и в начальной стадии заболевания в предшествующем опыте.

Рис. 3. Внеэритроцитарные формы *N. tadzhikistanica*, обнаруженные в период проявления клинических признаков заболевания (продолжение)
Fig. 3. Exoerythrocytal forms of *N. tadzhikistanica* detected in the period of manifestation of the clinical symptoms of disease (continued)

Таким образом, внеэритроцитарные одноклеточные формы *N. tadzhikistanica* удалось найти во внутренних органах за несколько дней до визуального обнаружения эритроцитарных форм. Внеэритроцитарные размножающиеся стадии обнаружены в начале заболевания и через 50 дней после появления первых паразитов в периферической крови, что позволяет предположить о параллельном развитии, а возможно и чередовании эритроцитарных и внеэритроцитар-

ных форм. Однако, на что мы считаем особенно важным обратить внимание, все внеэритроцитарные формы, обнаруженные нами, располагаются в плазме крови. Это позваляет нам высказать сомнение по поводу описания Трофимовым 1952, внутриклеточных форм развития систематически близкого паразита *N. equi.*

Предшествующие эксперименты показали нам, что в крови позвоночного хозяина в течение всего инкубационного периода присутствуют какие-то формы нутталлий, которые могут быть довольно легко выявлены биопробой на стерильных животных. Интересно было установить, способны ли эти формы в инкубационный период инвазировать клещей-переносчиков или нет. Иными словами, имеют ли они биологические различия с теми формами, которые мы обычно видим в эритроцитах в процессе переболевания, или биологически идентичны им.

Этот вопрос решался следующим образом. На песчанке, зараженной нутталлиями через нимф *H. anatolicum*, кормились две группы стерильных личинок *H. anatolicum* (воспринимающая инвазию фаза — личинки, передающая — нимфы). Первая группа личинок кормилась с 1-го по 5-й день, вторая с 6-го по 14-й день инкубационного периода, при длине инкубационного периода 16 дней. Затем нимфами, перелинявшими из этих личинок, заражались стерильные 2 спленэктомированные и 2 интактные песчанки.

При самом тщательном обследовании этих песчанок в течение 45 дней установить заражение нутталлиями не удалось. Последующее заражение подопытных песчанок показало, что они не обладают иммунитетом к *N. tadzhikistanica.*

Таким образом, клещи, питавшиеся на зараженной песчанке в икубационный период, не восприняли инвазии. Можно было думать, что в этот период очень мало паразитов, и они не попали с кровью в личинок. Для того, чтобы устранить это возражение, мы параллельно от песчанки, на которой питались личинки, в различные сроки инкубационного периода брали по 5—6 капель крови и вводили ее стерильным животным. Все песчанки, получившие иньекцию крови, взятой начиная с 4-го дня инкубационного периода, заразились нутталлиями. Личинки (а их кормилось на песчанке по нескольку сот в обеих группах) высасывали больше крови, чем мы брали для биопробы. И очень вероятно, что формы нутталлий, которые вызвали заражение стерильных песчанок, попадали в личинок, но, как мы видим, не инвазировали клещей, и клещи не передали нутталлий позвоночному хозяину.

Решение вопроса о биологическом различии или тождестве форм пироплазмид, паразитирующих в позвоночном хозяине в различные периоды инвазии, представляется весьма интересным с многих точек зрения. Поэтому мы приводим результаты нашего опыта, хотя, безусловно, он не может претендовать на окончательное решение вопроса, для этого необходимы повторные неоднократные проверки эксперимента.

Резюме

Проведенные исследования показали, что клещи передают инвазию позвоночному хозяину лишь после некоторого периода питания.

Boophilus calcaratus инокулирует *Piroplasma bigeminum* на 9-й день после подсадки, однако в одном опыте при подсадке большого количества клещей (свыше 20 000) получена передача инвазии на 4-й день после подсадки личи-

нок. *Nuttallia tadzhikistanica* переносится клещем *Hyalomma anatolicum* через 96 часов, а *Babesiella bovis* клещем *Ixodes ricinus* через 48 часов после подсадки.

Весь период от момента инокуляции паразита клещем до обнаружения паразитов в мазках периферической крови методом микроскопии, *P. bigeminum*, *B. bovis* и *N. tadzhikistanica* присутствуют в кровяном русле позвоночного хозяина и могут быть обнаружены биопробой.

У *N. tadzhikistanica* так же, как и у *P. bigeminum* имеются формы, способные размножаться вне эритроцитов в плазме крови. Чаще всего эти процессы идут во внутренних органах, богатых кислородом — легких, сердечной мышце, головном мозге.

Личинки *H. anatolicum*, питавшиеся в инкубационный период на песчанке, зараженной нутталлиями, не инвазировались и не передали в нимфальной фазе нутталлий стерильным песчанкам.

SUMMARY

Ticks transmit the invasion to the vertebrate host only after a certain period following the feeding. In most cases *Boophilus calcaratus* inoculates the *Piroplasma bigeminum* the 9th day after being placed on the host. *Nuttallia tadzhikistanica* is transmitted by the tick *Hyalomma anatolicum* after 96 hrs., and *Babesiella bovis* by the tick *Ixodes ricinus* after 48 hrs.

In the whole period, from the moment of inoculation the parasite by the tick till the detection of parasites in the peripheral blood smears by microscopic methods, *P. bigeminum*, *B. bovis* and *N. tadzhikistanica* are present in the blood stream of the vertebrate host and may be revealed by a biological test.

In *N. tadzhikistanica* as well as in *P. bigeminum* forms exist which are able to reproduction out of erythrocytes in the blood plasma. Most frequently the reproduction process occurs inside the blood capillaries of the internal organs.

The larvae of *H. anatolicum* feeding in the incubation period on *Meriones erythrourus* invaded by *Nuttalia*, fail to transmit the invasion in their nymphal phase to the parasite-free individuals.

The results of the study support the view about the existence of extracellular preerythrocytal forms in piroplasmidae.

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Notes on the extrusion and some other features of myxosporidian spores

Poznámky k vystřelování a k některým jiným vlastnostem spor myxosporidií

Recently we are noting an increasing interest in *Myxosporidia*. Their role of an important fish pathogen is reflected by a series of recent papers on the epidemiology of their most significant representatives (e.g. the whirling disease — Uspenskaya 1957, Hoffman and others 1962); the incessant ichthyoparasitological research has brought forth the first taxonomic monograph on *Myxosporidia* since the time of Kudo, i.e that of Shulman 1962; their cytology, taxonomy and evolution begin to attract attention again (Grassé 1959, Cheissin et al. 1961, Lom and Vávra 1962, Corliss and Levine 1963, Lom and Vávra 1964). In spite of the increasing knowledge gathered in the research of these remarkable protozoans, the most interesting and with regard to infection, the most important stage of their life cycle, the spore, has been rather neglected. We have but spare data on its composition, mode of germination and viability.

Extrusion of the polar capsule

Since the time of Thélohan 1895 who reviewed different chemicals used with success to induce the filament extrusion (in his experiments, K_2CO_3 and Na_2CO_3 were most effective, inducing extrusion in 11 myxosporidian species) no progress has been made in elucidating the interesting biological process of the myxosporidian extrusion. Merely some new agents inducing the extrusion in certain myxosporidian species have been added — e.g. 5% phenol (Laird 1953). Out of these chemicals, we have found the H_2O_2 to be most efficacious. However in many species only its concentrated 30% solutions release 100% of the capsules, this being a great difference when compared to the microsporidians. If used in more diluted solutions, its effect is far below 100%, and very often the extrusion can be observed only in the very moment when the drop of the mixture dries up. Perhaps only the dessication accomplishes the change, initiated by hydrogen peroxyde, and releases the filament (probable osmotic effects or simple pressure have been experimentally eliminated as the causing agents). However H_2O_2 is ineffective in many species of myxosporidians.

As to the similar extrudible organelles of other groups of organisms, acceptable theories on the mechanism of extrusion have been presented — for *Microsporidia* see Lom and Vávra 1963 and for nematocysts of coelenterates — papers by Yanagita. Let us consider the results of the latter author, since the morphology and even morphogenesis of myxosporidian polar capsule reveal remarkable similarity with coelenterate's nematocysts (Lom and Vávra 1964). According to this Japanese author, the nematocyst is equipped by a "stopper" mechanism. After it had been disintegrated by a certain "trigger" process, it opens the way to the release of energy, persisting still in the ripe nematocyst which expulses the filament. The "stopper" is probably a protein structure consisting of two components — the first one being probably keratin, since the extrusion is provoked by sodium thioglycollate in low concentrations and the second one a protein gel which can be destroyed by the action of trypsine or in solutions with extreme pH. The stopper mechanism ceases to suppress the intracapsular tension as soon as one of the two components has been disintegrated, and the preexisting tension releases the filament.

As experimental object we choose *Myxobolus muelleri* Bütschli from the gill plates of *Abramis brama*. We tried the chemicals used by Yanagita without success: trypsin and pepsin in different concentrations and in varying pH, sodium thioglycollate in concentrations up to M/4 and extreme values of pH (acids and bases) failed to produce the extrusion.

With the idea of the protein stopper mechanism in mind, we employed the urea. When applied in saturated solution — one drop to one drop of fluid with spores — it causes the extrusion within 30″ to 2′ in 100% of fresh spores. In spores stored for some time the extrusion may occur after several minutes of the action of urea and not in all spores. For the time being, the spores of all species we have had the occasion to investigate, belonging to genera *Myxobolus, Myxosoma, Thelohanellus, Henneguya, Myxidium, Zschokella, Chloromyxum* and *Sphaeromyxa* have been found sensitive. In weaker solutions, the effect of urea decreases rapidly.

On applying the urea, the spores shrink considerably (but not the polar capsules within them!) due to the highly hypertonic medium — a saturated solution at +23°C has the osmotic pressure of about 255 atmospheres. Afterwards, the filament extrudes. Then the spore assumes its normal shape as if the valves had ceased to function as semipermeable membranes; this occurs also when the extrusion is hampered. Viewed in the phase contrast, the polar capsules are quite clear and so is the sporoplasm. Just before the extrusion, the capsule appears a hue dimmer as if the structure of its content were beginning to alter. After the expulsion of the filament, the inner space of the spore begins to communicate with the outer medium and the sporoplasm turns dark — a sign of alteration due to urea. If a stain is diluted in the urea the spore content stains after the extrusion.

The way in which urea causes the extrusion is not quite clear. It is not the effect of the osmotic pressure — solutions of NaCl or sucrose of comparable osmotic value are not effective; on the contrary, they hamper the extrusion. Urea is well known to be a denaturating agent for proteins, especially for those of the globular type, in which it causes desagregation of the protein molecules and their decomposition to smaller entities while reacting with the hydrogen bridges between the peptid chains. Could not

the urea in polar capsules also disintegrate a sort of "stopper mechanism" consisting of protein? The morphological structure which would correspond to such a stopper, is situated on the apex of the capsule on the spot where the capsule is attached to the channel in the shell valve through which the filament extrudes. This cap can be observed on polar capsules of many species e.g. of the genus *Myxobolus* (C h e i s s i n's electron microscopic observations from 1961, personal communication of S h u l m a n) and is perhaps identical with the structure on the apex of the capsules in our material of *Myxobolus muelleri*. This structure stains intensively with bromphenol blue protein method.

Let us continue in comparing some similar and different features of the extrusion process in *Myxosporidia* and *Microsporidia*. We shall not deal here with the different functions of their filaments — for *Microsporidia* a detailed account has been given recently (L o m and V á v r a 1963). In *Myxosporidia*, anchoring is probably the only function of the filament — we have been able to observe its great sticking capacity.

Myxosporidian polar capsules approach considerably the nematocysts of coelenterates, if we consider the "stopper" — the protein cap-like structure on their apex. Having disintegrated this cap, urea may activate also the mass within the polar capsule and release the filament or, more probably, the disintegration of the cap itself brings about changes resulting in the extrusion. The stopper hypothesis is the more probable, as the extruded capsules — like those of the nematocysts — have a much smaller volume than the intact ones (Pl. II 5). Thus they should not increase their volume by imbibition of water, as supposed for *Microsporidia* (L o m and V á v r a 1963), to replace the material driven into the filament (the myxosporidian filaments have closed tips!). The firm walls of the capsules are probably also of considerable elasticity.

So far, microsporidian spores have not been found to be sensitive to urea (neither to sodium thioglycollate). The McMannus positive structure in the microsporidian spore can hardly be held for the stopper; it persists in its position even after the extrusion had been completed. The spore wall is permeable for water, at least to a certain degree, as we have clearly seen in our experiments with the spores of *Plistophora hyphessobryconis*, *Nosema* sp[1]. and *Thelohania chironomi* exposed to hypertonic solutions. The volume of their posterior vacuoles decreased in weak concentrations of sucrose or NaCl, reassuming the original state when returned to water. Therefore the anterior pole of the spore may not be the only place through which water can contact the polaroplast, after the McMannus positive cap had been invaded, as believed recently. More probably, the cap is an easy-to-penetrate point of the spore wall, the polaroplast being able to receive water through the whole surface of the shell. To say, whether the agent, capable to initiate the swelling of the polaroplast, can reach it through the mass of the cap or through the spore wall, would be a mere speculation.

Difference also exists between the ultrastructure of the polaroplast, which is of a finely granular structure in the inactivated state whereas in the activated state it has a laminar structure (H u g e r 1960, L o m and V á v r a 1961), and the mass within the polar capsule which appears homogeneous in the electron microscope (L o m and V á v r a 1964).

[1] Identical with *Nosema* sp. of C o r t (J. Parasitol. 1960).

The independence of the extrusion of the polar capsules from the osmotic pressure of the environment, demonstrated by their expulsion in solutions with osmotic pressure of 255 atm., would seem to keep *Myxosporidia* apart from *Microsporidia*. As to the latter it was found that the extrusion was made impossible if the osmotic pressure of the environment surpassed some 50 atm. However, in the case of urea, capable to dissolve the proteins of the spore, the real osmotic pressure must not necessarily be so high as would correspond to the calculated values. If we saturate the urea solution with NaCl, no extrusion occurs; the capsules begin to discharge only when the concentration of NaCl in the saturated solution of urea decreases to a degree corresponding to the osmotic pressure of 70 atm. Similar results have been obtained with the spores of *Myxobolus* activated by hydrogen peroxyde in a concentrated saccharose solution. We may therefore conclude that the highly hypertonic solution may alter the active mass within the capsule and hamper the extrusion.

All these conclusions on both the microsporidian and myxosporidian extrusion may be regarded as first steps in explaining the mechanism causing the expulsion of polar filaments.

Morphology of the extruded filaments

Urea-extruded spores of *Myxobolus muelleri* were embedded in araldit and the ultrathin sections were examined in the electron microscope. Urea does not destroy the components of the spore, but the sporoplasm is shrunken and detached off the spore walls. The extruded filaments are hollow tubes (Pl. I 3, F), not circular in the cross section. In the inverted filament, still in the capsule, the cross section has the form of the figure 8, or is irregular, as the filament is spirally twisted; subsequently in an everted, extruded state, it is either a ring with two folds, corresponding to the notches in the cross section of an inverted filament, or it is also irregular. The inner surface of the filament is smooth, the outer one bears the remnants of the substance which had previously filled the lumen of the filament. The filaments penetrate the massive anterior edges of the shell (Pl. I 1, 2, 4) by a channel-like opening. At the inner end directed towards the capsule, the walls of these channels are formed by both valves; towards outside the channel continues like a narrow opening through the edge of only one of the valves. In the inextruded state, this channel is filled by a material which may be identical with the "cap" of Cheissin et al. 1961 and representing probably the stopper mechanism of the capsule. The remnants of this material encircle also the wrinkled tube of the extruded filament in the channel-like opening in the edge of the shell valve.

The intercapsular appendix between the polar capsules, serving for specific determination, is formed by the joint anterior edge of the shell valves (Pl. I 2, A) reaching more or less backwards between the capsules.

The extruded filaments are resistant to tryptic and peptic digestion.

The shell valves

In ripe spores of *Myxobolidae* the shell consists of two relatively thin valves with massive edges. The shell of each valve is composed of two layers which divide at the borders of the valve and enfold the finely granular

material forming the greatest part of the mass of the shell's edge. It is evidently the remnant of a transformed cytoplasm of the valvogenic cell. With the exception of the edges this material is disappearing; the two thick layers of the shell originated evidently from the pellicle of the valvogenic cell and the transformed cytoplasm has been preserved only in the edges. On the inner side of the shell there is a thin electron dense layer, reaching also slightly between the two valves of the shell at the edge.

The shells are wholly dissolved by alkaline hydrolysis (in NaOH by autoclaving, in 30% KOH after 9 minutes of boiling). They are resistant to tryptic and peptic digestion, and also to the usual histological stains. They are also only weakly stained by the mercury-bromphenol blue method for detection of proteins; however, if before staining we expose the isolated shell valves to alkaline hydrolysis (30% KOH at room temperature) they stain quite distinctly (Pl. I 6), especially the brims. The coupled tetrazonium reaction for tyrosine, histidine and tryptophan (Burston 1955) gave only a fine tinge to the shells, the azo-coupled reaction for tyrosine (Glenner and Lillie 1959) gave no result. The performic acid-alcian blue method for SS-groups (Adams and Sloper 1956) gave also no result; the DDD reaction for SH-groups (Barnett and Seligman 1952) yielded a weak positive result. Methods for polysaccharides: PAS and also the alcian blue method for acid mucopolysaccharides (Stedman 1950) were entirely negative. So was the chlorzinciodine stain for chitin (Pearse 1961).

In *Microsporidia,* however, the last cited staining method gave positive results with spores of species quoted on p. 323 (in agreement with Vávra — personal communication). The protein methods we used were negative in these microsporidian spores; also the alkaline hydrolysis did not dissolve them. We suppose the myxosporidian shell to be proteinous and composed of a very resistant kind of protein. Most probably it is not keratin, contained e.g. in the denticles of *Trichodina,* and accordingly it shows no optical anisotropy. Further research of the chemical composition will have to face the problem of setting apart the thin shells and the thick edges, containing probably different proteins.

The resistance of spores

Previous authors reported the short longevity of spores outside their hosts: Thélohan 1895 and Linton 1891 found the spores of different myxosporidian species to be viable for 1—2 months and 8—10 days respectively; Auerbach 1909 — *Myxidium bergense* 10 days, *Myxobolus aeglefini* 24 days; Bond 1938 — *Myxobolus subtecalis* 15 days, *Myxidium folium* 20 days and only *Myxobolus bilineatum* for an almost unlimited time.

Recent authors show, on the contrary, that the spores can maintain their viability for a very long period, e.g. Bauer 1959 reports a period up to 12 years in *Myxosoma cerebralis,* Hoffman et al. 1962 up to 22 months in the same species.

Our experiments have revealed that the spores of most species of the genus *Myxobolus* and *Myxosoma* may be preserved at +4°C for more than one year. We keep the spores in test-tubes of the inner diameter of 6 mm. in a small quantity of water; the better the spores have been cleared of the remnants of the tissue, the longer they preserve their viability, the decay

of the tissue damaging them considerably. *Henneguya* is more sensitive than *Myxobolus*.

Furthermore, we have corroborated the old data on the extreme resistance of spores towards dessication or alcohol. K u d o 1919 stated that the spores of *Myxobolus discrepans*, fixed in acid alcohol and kept in 95% alcohol, were capable of extrusion by means of KOH still after 5 months. T h é l o h a n 1895 found that spores of *Myxobolus elipsoideus*, dried for several years, could be extruded by K_2CO_3.

Myxobolus muelleri spores were capable of extrusion in 100% urea even after a 2 hours' stay in 70% alcohol; after 15 hours, more than 60% of the spores extruded and only a stay longer than one day prevented the extrusion. Spores of the same species extruded still after having been preserved for 35 days as a dry smear.

Summary

A standard method for filament extrusion of myxosporidian polar capsules by urea is described. In connection with the effect of urea, some ideas concerning the mechanism of extrusion and its comparison to that in microsporidian spores are expressed.

The extruded filaments were examined in the electron microscope. The situation of the discharging channels of filaments and of the intercapsular appendix in the apex of the spore, is described.

Evidence of the protein nature of the shell is presented.

Spores can be stored for experimental as well as taxonomic purposes for longer periods in a refrigerator.

SOUHRN

Je popsána metoda vystřelování polárních váčků ve sporách myxosporidií pomocí močoviny. Tato metoda může nalézt velké upotřebení pro taxonomickou charakteristiku jednotlivých druhů. Byl diskutován možný mechanismus účinku močoviny na polární váčky a mechanismus vystřelování polárních vláken u myxosporidií vůbec, a provedeno srovnání se sporami mikrosporidií.

Vystřelená polární vlákna byla pozorována v elektronovém mikroskopu. Je popsán způsob, jakým vlákna vycházejí ze spory ven zvláštními kanálky; interkapsulární výrůstek, důležitý pro systematiku myxobolidů, je ztlustlým okrajem spory mezi kanálky pro vlákna.

Skořápky spor jsou bílkovinného charakteru.

Spory mohou být k pokusným i taxonomickým účelům skladovány po značnou dobu v ledničce.

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EXPLANATION OF PLATES I-II

Fine structure of the spores of *Myxobolus muelleri*, extruded artificially by urea

1: Section through the anterior thick edges of the shell valves, with transversely cut discharging channels of the filaments, each one situated separately within the edge of one valve. One filament has been discharged, the other is still closed by a "stopper" (S). At the top and the bottom, two parts of transversely cut spores are situated showing thin shell valves with thick edges.

2: Oblique section through the anterior apex of the spore with both filaments extruded. A — parts of the valve edges extending backwards into the spore to represent the intercapsular appendix.

3: At left a part of the spore, at right three cross sections of extruded filaments, to the outer surface of which adheres the material, formerly filling the lumen of the inverted filament and causing the stickiness of the filament.

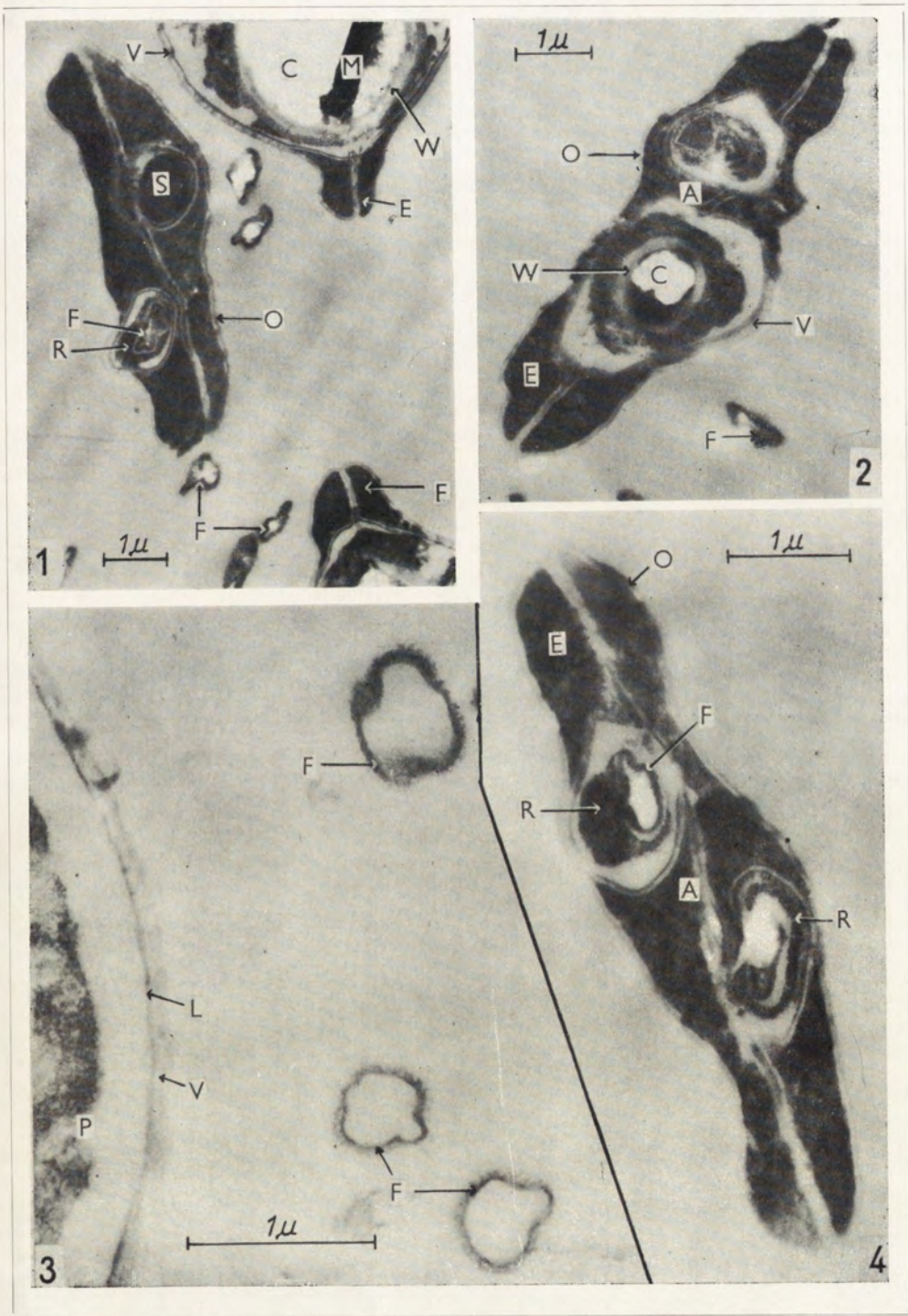
4: Another section through the spore with extruded filaments. Rennants of the stopper well visible.

[F — wall of the sectioned hollow filament, V — shell valve with inner layer L, E — thick edges of the valves filled with material originating from the plasm of the valvogenic cell and covered with one layer of the shell valve O, P — shrunken protoplasma, C — extruded polar capsule, W — its wall, M — remnants of the intracapsular material, R — remnants of the "stopper" material]

Light microscope pictures of myxosporidian spores

5: Spores of *Myxobolus muelleri* with extruded (A) and inextruded (B) polar capsules. Phase-contrast, oil immersion lens.

6: Isolated spore shells of *Henneguya psorospermica*, stained by the mercury-bromphenol blue method for proteins. Oil immersion lens.



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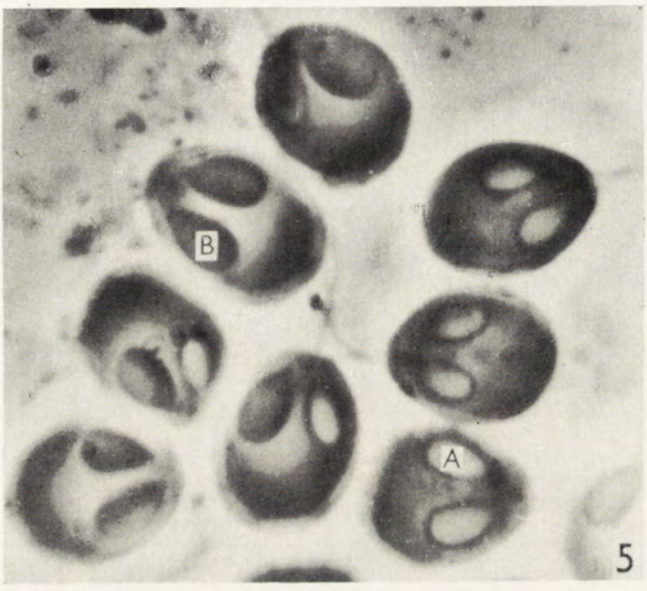

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Cytophotometric study of RNA content in the macrogametogenesis of two rabbit intestinal coccidia *Eimeria magna* and *E. intestinalis*

Цитофотометрическое исследование количества РНК в макрогаметогенезе двух кишечных кокцидий кролика *Eimeria magna* и *E. intestinalis*

Nucleic acids, their role in the cell metabolism as well as the methods of their determination are a most attractive point concentrating the attention of numerous investigators. The branch of science dealing with nucleic acids undergoes a rapid development so that almost every new investigation provides innovations into our knowledge which seemed to have been stable yesterday. In this respect quantitative methods of investigation are of great importance (Casperson 1936, Brachet 1942, for a review on this subject see Pevzner 1963).

Especially important is the role of quantitative methods when studying nucleic acids in growing cells. The studies available do not give, however, a uniform reply as to what happens, for example, with RNA during the cell growth. Does it increase or decrease? In the majority of investigations made, only qualitative methods of histo- and cytochemistry were used. One group of authors reported an increase in RNA content (Bogomolova 1959, Pavlova 1959), while the others found a decrease in RNA amount in the course of oogenesis (Brachet 1941, Pasteels 1948, Fautrez 1950, Vakaet 1950, Kiknadze 1955, Makarov 1956, Petrova 1956, Choubareva 1957 and others).

However, in case when quantitative methods, both histochemical and biochemical, were used the increase in RNA content was invariably shown during the growth of oocytes (Brachet 1942, Flax 1951, Steinert 1951, Osawa and Hayashi 1953, Eisenstadt et al. 1964).

The growth of a macrogamete of *Coccidia* resembles in a certain degree that of an oocyte of *Metazoa*. Yet, no quantitative studies of macrogametogenesis of coccidia have been carried out. However, the comparison of two similar processes (oogenesis and macrogametogenesis), occurring in *Metazoa* and in *Protozoa*, would give much opportunity for scientific speculation. The present study represents the first attempt of a quantitative investigation of RNA content in growing macrogametes of coccidia belonging to the genus *Eimeria*.

The distribution of RNA in the life cycle of rabbit coccidia *Eimeria magna* and *E. intestinalis* was thoroughly studied by Cheissin 1958, 1960. Using

methods of qualitative cytochemistry Cheissin found the cytoplasmic basophilia of the macrogamete, mainly due to RNA, to decrease with the cell growth. However, the question of the changes in RNA amount during the macrogametogenesis remained still non-settled. Hence arose the necessity of quantitative estimation of RNA in the growing macrogamete.

It was impossible to presume basing on the cytochemical data only in which direction the initial content of RNA would change during macrogametogenesis of *Coccidia* which are known to be highly specialized intracellular parasites. The obligatory intracellular parasitism represents an extreme example of the metabolic dependence of the parasite on the host (Moulder 1962). Keeping this in mind, one could expect that RNA in a growing macrogamete would change in its own peculiar way. Therefore, a quantitative study of RNA in growing macrogametes of *Eimeria* seems to contribute much also to the problem of intracellular host-parasite systems.

Material and methods

Two groups of rabbits sensitive to coccidiosis were employed in the experiments. The rabbits were fed with sporulated oocysts of *Eimeria magna* and *E. intestinalis*, resp. Animals were killed on different days of gametogenesis so that parasite macrogametes of various size, i. e. of different age, were found on the slides.

As the fixative, Zenker's fluid was used. Paraffin 7 μ sections were stained with gallocyanin-chromalum (Pearse 1960); sections treated with crystalline ribonuclease were used as a control.

The applicability of gallocyanin-chromalum method for the aim of quantitative histochemistry was recently reported by Ovchinnikova i Selivanova 1964.

The measurements were performed on the microspectrophotometer MUV-4 by the scanning method in λ 579 mμ.

The estimation of RNA content was carried out on the sections, and the total amount of RNA in relative units was obtained from calculation of RNA in the whole volume of the macrogamete, assuming that the latter would have a ball-like form (not long before the fertilization of the macrogamete its form changes for ovoid one).

Only those macrogametes were used for a photometric estimation that were cut exactly across the nucleolus, which occupies the central position in the macrogametes of species investigated (Cheissin 1947, 1948). This allowed to measure easily the radius of the macrogamete which was taken from the photomicrographs of corresponding cells.

The present work represents a cytophotometric study of a cytoplasmic RNA only.

The total quantity of RNA in the cytoplasm can be expressed by the following formula

$$Q = \frac{DV_{cyt}}{h}$$

where Q is the total quantity of cytoplasmic RNA, D — optical density, V_{cyt} — volume of the cytoplasm, h — thickness of the section.

But:

$$V_{cyt} = V_{cell} - V_{nucleus}$$

It is known that

$$V = \frac{4}{3}\pi R^3$$

therefore

$$V_{cyt} = \frac{4}{3} R^3_{cell} - \frac{4}{3}\pi r^3_{nucleus} = \frac{4}{3}\pi (R^3_{cell} - r^3_{nucleus})$$

where R_{cell} — radius of the cell, $r_{nucleus}$ — radius of the nucleus.
Finally:

$$Q = \frac{D}{h} \cdot \frac{4}{3}(R^3_{cell} - r^3_{nucleus})$$

The photometry of RNA content in oocysts of *Eimeria* was not performed.
All macrogametes measured were then distributed into five classes of different size, according to the values of their cross-section areas, for either species separately. Each size class differed from the neighbouring one by equal number of area units. The photomicrographs of each class of macrogametes are shown in Pl. I-II. The data obtained were treated statistically.

Results

The results obtained from the cytophotometry of mactogametes are represented in the Tables 1 and 2.

Table 1

Results of the photometric estimation of RNA in the process of macrogametogenesis of *Eimeria magna* in relative units $\times 10^{-6}$

Size classes of macrogametes	The number of cells measured	RNA quantity (Q) M±m	Volume of macrogametes M±m	Concentration of RNA (Q/V)
I	44	16.32±1.13	24.63±1.50	0.6626
II	40	41.38±3.51	63.87±2.30	0.6478
III	31	43.66±3.09	103.00±4.27	0.4238
IV	27	75.27±5.40	161.10±4.80	0.4672
V	11	134.0±10.60	229.50±18.00	0.4840

As shown in Tables 1 and 2, the amount of RNA in macrogametes of both species increases progressively with their growth. The differences between the neighbouring size classes appeared statistically significant with probability equal to 99.9%, except two cases: 1. between classes II and III of *E. magna* (Table 1), 2. between classes IV and V of *E. intestinalis* (Table 2). In the

former case the difference is not statistically significant, while in the latter one, the difference may be assumed as statistically significant with probability equal to 95%.

Table 2

Results of the photometric estimation of RNA in the process of macrogametogenesis of *Eimeria intestinalis* in relative units $\times 10^{-6}$

Size classes of macrogametes	The number of cells measured	RNA quantity (Q) M±m	Volume of macrogametes M±m	Concentration of RNA (Q/V)
I	43	3.67±0.26	5.75±0.51	0.6380
II	44	15.12±0.92	20.30±0.88	0.7440
III	37	30.04±1.70	39.20±1.25	0.7665
IV	25	38.70±0.60	62.73±1.70	0.6171
V	15	54.00±5.10	92.50±2.40	0.5833

The results obtained are also shown in the Figures 1 and 2. In both species, the increase in RNA content occurs but its course is different in *E. intestinalis* and in *E. magna*. In the former species the curve reflecting the amount of RNA rises uniformely, while in the latter, a pronounced delay is seen at the beginning phase of the macrogamete growth.

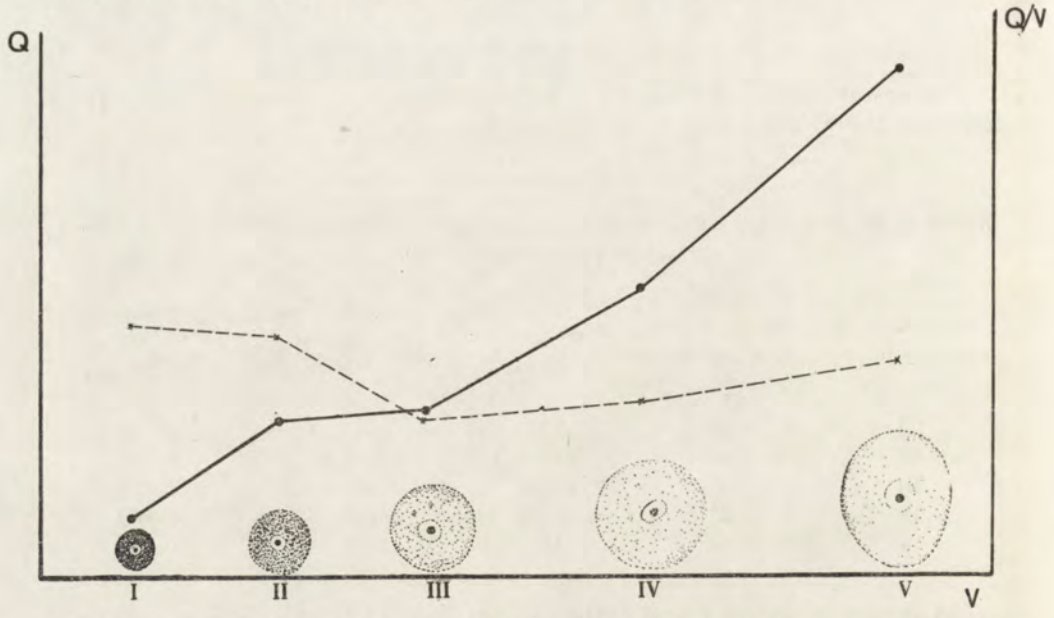

Fig. 1. Changes in the cytoplasmic RNA content in the growing macrogametes of *E. magna*. V — volume of the cell, Q — RNA quantity per cell, Q/V — RNA concentration. Continuous line represents changes in RNA quantity per cell, dotted line represents changes in RNA concentration. Latin numerals show numbers of size classes of macrogametes

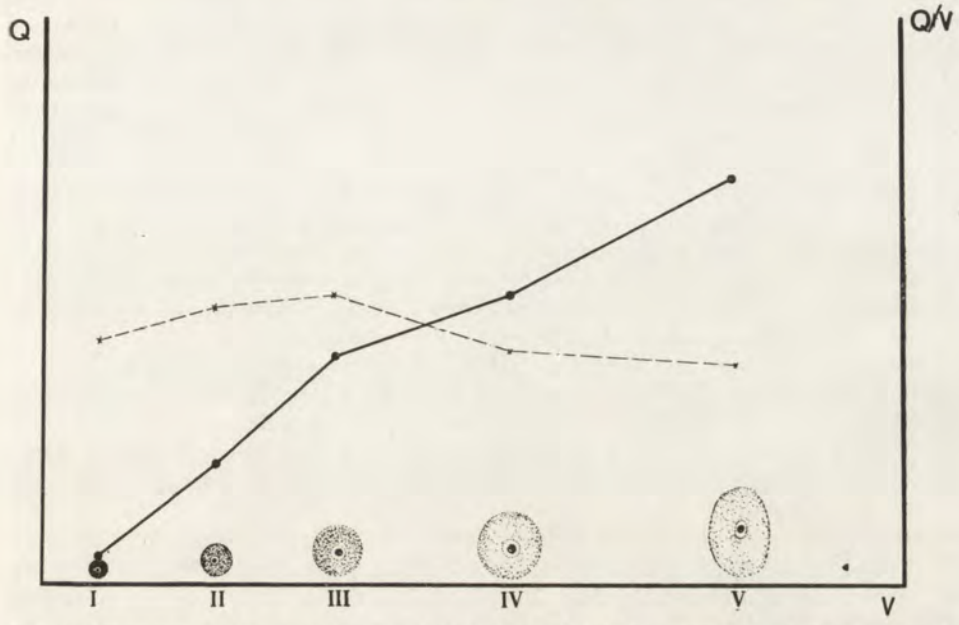

Fig. 2. Changes in the cytoplasmic RNA content in the growing macrogametes of *E. intestinalis*. Lettering the same as in Fig. 1

As the cell enlarges, the concentration of RNA per unit of volume decreases. This indicates that the growth of the macrogamete is faster than RNA synthesis.

Discussion

The general pattern of basophilia of macrogametes of *E. intestinalis* and *E. magna* found in sections (Cheissin 1958, 1960) seems to reflect the RNA concentration curve obtained from our cytometric measurements (Figs. 1 and 2). Judging by RNA stainability on the sections, one might come to an erroneous impression that the RNA content is decreasing during macrogametogenesis of *Eimeria* (Pl. I—III).

However, the comparison of results obtained from cytochemical and cytophotometric studies of *Eimeria* has shown how prompt is the evaluation of quantitative changes in the RNA content basing only on the intensity of cytoplasmic basophilia. Our results indicate that though the RNA concentration falls, as the cell grows, the total amount of RNA in the cytoplasm of macrogamete increases continuously.

Observers who reported that RNA content increases during oogenesis, consider RNA to be necessary for synthesis of the yolk proteins (Brachet 1941, Fauré-Fremiet et al. 1950, Fautrez 1950, Vakaet 1950, Petrova 1956). At the same time, quantitative measurements of RNA in oogenesis of amphibians, eggs of which are known to be rich in yolk, provided quite different results. Osawa and Hayashi 1953 performed quantitative biochemical estimation of RNA and protein contents in growing oocytes of *Triturus pyrrhogaster* and the data observed were compared with the histoche-

mical picture of the same subject. It appeared that the amount of RNA per a cell increased progressively with the growth of the latter in the early phase of oogenesis. However, beginning from a certain period of oogenesis, the value remained almost constant in spite of increase in the cell volume. On the other hand, the concentration of RNA (its amount per mm^3) fell sharply during the early phase and then declined gradually.

The data of the Japanese authors confirm the statement postulated earlier by Brachet 1942. Young oocytes contain always large amount of RNA in cytoplasm and in nucleoli. During vitellogenesis the RNA content is "diluted" in the continuously increasing oocyte volume. However, the RNA content also increases during the course of oogenesis, but in the later stages the growth of the oocyte is faster than RNA synthesis.

Recently, a quantitative ultra-violet measurement of the RNA amount in oogenesis of *Glossiphonia* was made (Eisenstadt et al. 1964). The quantity of RNA was shown to increase continuously as the oocyte grew. Thus, results available from quantitative measurements indicate that in oogenesis of *Metazoa* the RNA content of the oocyte becomes much higher, as compared with its initial amount.

Data concerning the cytochemical studies of *Protozoa* are the following.

Patillo and Becker 1955 reported different stainability of growing macrogametes of chicken coccidia *Eimeria brunetti* and *E. acervulina,* obtained with histochemical methods; a strong basophilia of young forms was strongly reduced in older macrogametes. Cheissin 1958, 1960 who studied nucleic acids in life cycles of rabbit coccidia *E. intestinalis* and *E. magna* by means of a qualitative cytochemistry, found similar changes in cytoplasmic basophilia of macrogametes. However, this author, like Patillo and Becker, did not take into account the changes in RNA content of the macrogamete during its growth and development.

Scholtyseck 1963 reported a continuous decrease in the cytoplasmic RNA content during the macrogametogenesis of *E. maxima* (chicken coccidia). The appearence of the peripheral protein granules of the macrogamete at the expense of the cytoplasmic RNA was assumed to account for this decrease. The author's conclusion was drawn, however, only as a result of qualitative cytochemical observations.

Stein 1960 examined the distribution of RNA in the life cycles of some gregarines. The material stained with methyl green—pyronin, after Brachet, was examined. The author came to conclusion that the cytoplasmic RNA content decreased as the gamont grew. Just the fading of the cytoplasmic basophilia was taken for a real decrease in the RNA amount.

The data reported in the literature as well as our own results prove the necessity of serious precautions, when estimating the quantity of RNA in a growing cell basing on the qualitative, even the most convincing, cytochemical picture.

As pointed out above it was difficult to predict the increase in the RNA content during the macrogamete growth because of the continuous drop in the intensity of the cytoplasmic basophilia. It might be thought that the adaptation of the parasite to its rather peculiar life in the host (intracellular parasitism) would have influenced the parasite's metabolism. Indeed, the very existence of such an influence has been shown in some cases: malaria parasites (Moulder 1962), leishmania (M. Lwoff 1940, A. Lwoff 1944, Kalli-

nikova i Roskin 1963). Hence, leishmanial forms of *Schizotrypanum cruzi*, the only reproducing forms of the parasite within the vertebrate host, appear the poorest in RNA. Kallinikova i Roskin suppose that in this case substances required for the growth and multiplication of the parasite may by synthesized by the host's cells.

Our results, however, allowed to state that a parasitic mode of life failed to influence the ribonucleic metabolism of the two *Eimeria* species and that the metabolism is similar to that in oogenesis of free-living *Metazoa*, if judging from results of quantitative studies.

A question arises as to the role of that tremendous increase of RNA at the end of macrogametogenesis, for this intracellular parasite.

To answer this question, it would be desirable to refer once more to data concerning oogenesis of *Metazoa*. The fact that no synthesis of RNA occurs during early stages of cleavage, made Brachet 1947 to postulate that a fertilized egg might have an extranuclear storage of nucleic acids. Histochemical studies have demonstrated that just this RNA which is stored is later utilized by the embryo in the course of its development. This fact accounts for the increase in the RNA content during oogenesis which is to be used not only for yolk protein formation during oogenesis but also for the developing embryo. Similarly, the RNA content of the macrogamete may have been used not only for the formation of protein granules during macrogametogenesis. Soon after fertilization the zygote (oocyste) of *Eimeria* is discharged from the host's organism into the external medium where it undergoes sporulation. The exogenous oocyste represents a closed system, the sporulation must have been accomplished at the expense of the RNA accumulated during the intracellular existence of the macrogamete.

Despite a great importance of the quantitative studies of RNA performed, we must still keep in mind that not the whole amount of the RNA measured is metabolically active. Although quantitative cytochemical methods can give an idea of the RNA content of the macrogamete they yield no precise information about the rate of protein synthesis which occurs at the stages of both macrogamete and oocyste. Thus the necessity of further autoradiographical studies of these stages is obvious. Besides, changes in RNA content in the nucleolus of the growing macrogamete require an additional examination.

In conclusion, we would like to discuss the question concerning species differences in the cytoplasmic RNA quantity of macrogametes between *E. intestinalis* and *E. magna*.

The increase in RNA content was shown for macrogametes of either species. As follows from Fig. 2, the curve reflecting changes in the RNA quantity of *E. intestinalis* macrogametes rises evenly. In the case of *E. magna* (Fig. 1), however, a delay in RNA increase can be seen between classes II and III. Then, after the delay, the amount of RNA becomes rising regularly. As pointed out by Cheissin 1947, the macrogamete of *E. magna*, on the contrary to that of *E. intestinalis*, soon after its establishment within the host's cell in the intestinal epithelium, changes its location for that in tunica propria (Pl. I—II). This change, followed by the adaptation to the new life conditions in tunica propria, as compared with those in the intestinal epithelium, might account for the delay in the increase of the RNA amount in *E. magna* which occurs at the first half of the macrogamete development.

Summary

A quantitative study of the cytoplasmic RNA content in growing macrogametes of two rabbit intestinal coccidia — *Eimeria magna* and *E. intestinalis* — was performed on the microspectrophotometer MUV-4 by the scanning in λ 579 mμ.

In spite of a sharp decrease in the intensity of the cytoplasmic basophilia, due to RNA, found with cytochemical methods in the growing macrogametes, the total quantity of RNA appeared to increase during macrogametogenesis, the highest value being measured just before the fertilization and formation of the oocyst. Similar results were obtained for both species studied.

Резюме

Методом сканирования в длине волны 579 ммк на микроспектрофотометре МУФ-4 проведено исследование цитоплазматической РНК в процессе макрогаметогенеза двух кишечных кокцидий кролика *Eimeria intestinalis* и *E. magna*. Оказалось, что несмотря на ослабление базофилии цитоплазмы, обусловленной РНК, в процессе роста макрогаметы, количество РНК в ней неуклонно возрастает, достигая максимума перед оплодотворением и образованием ооцисты. Данные для обоих исследованных видов кокцидий совпадают.

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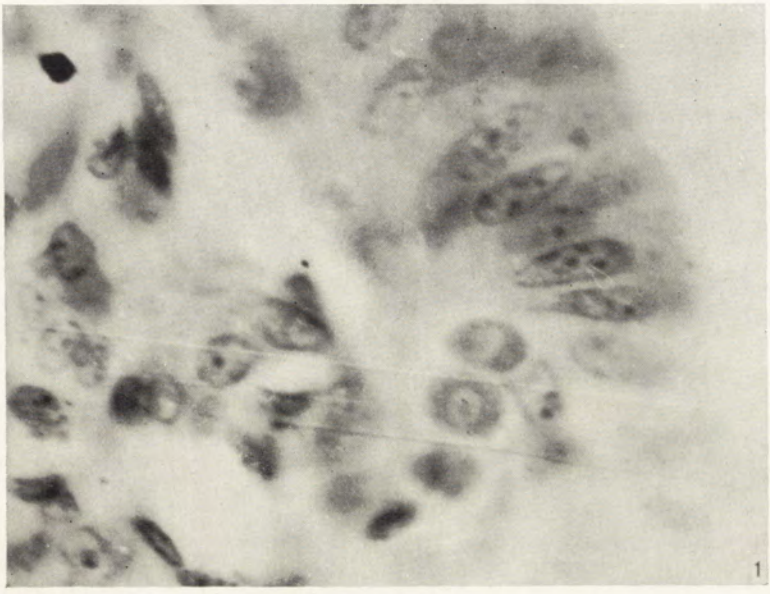
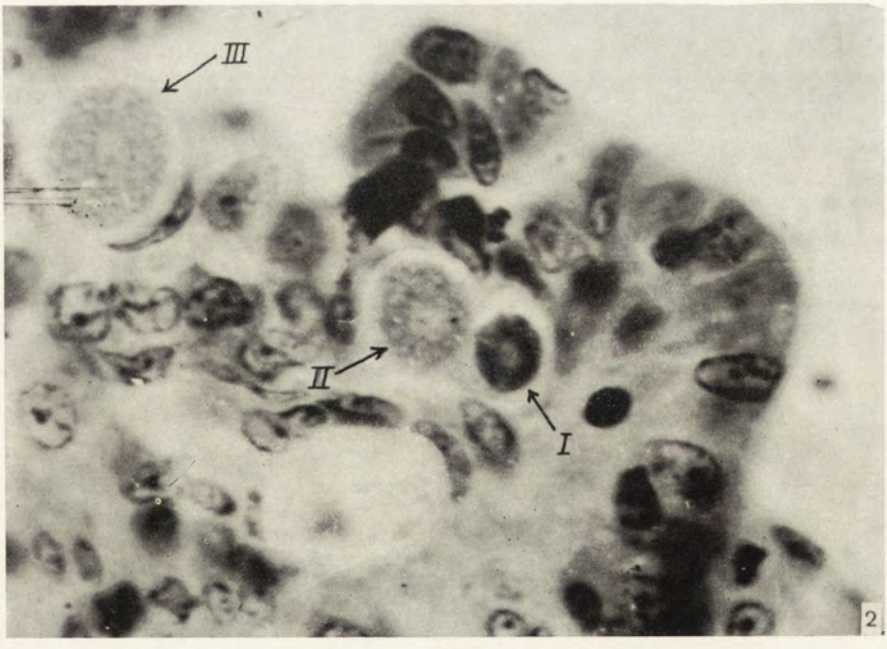
EXPLANATION OF PLATES I—III

1—4: Photomicrographs of *E. magna* macrogametes belonging to different size classes; 1 — macrogametes of the class I (the very beginning of leaving the tunica propria), 2 — those of the classes I, II and III, 3 — those of the classes II and IV, 4 — a macrogamete of the class V.

5: Photomicrograph of macrogametes of *E. intestinalis* belonging to different size classes.

[All stained with gallocyanin-chromalum, ×3135]





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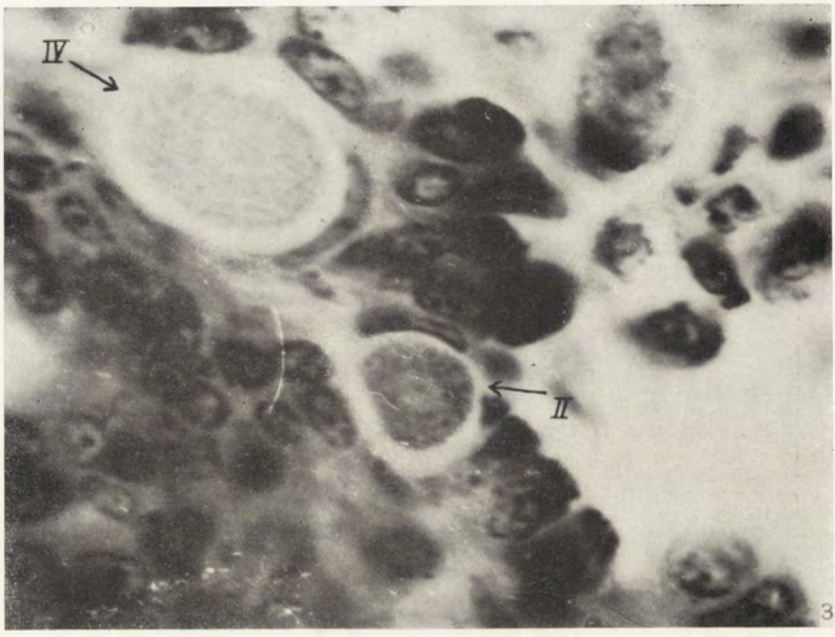

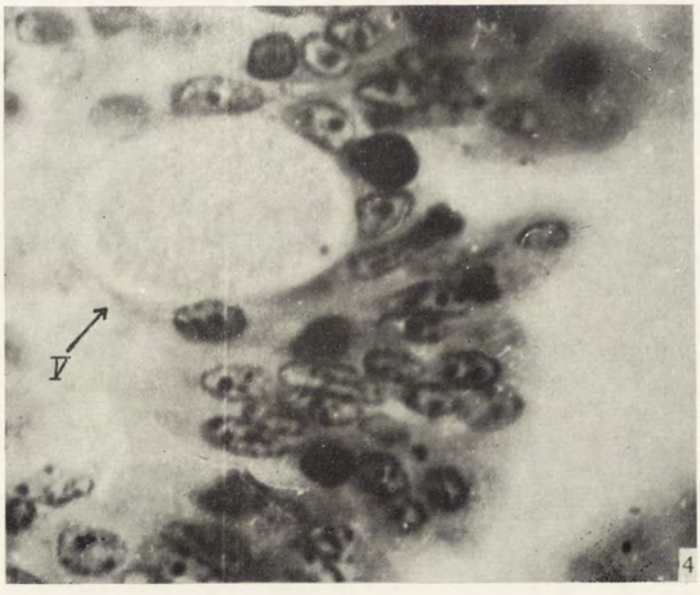

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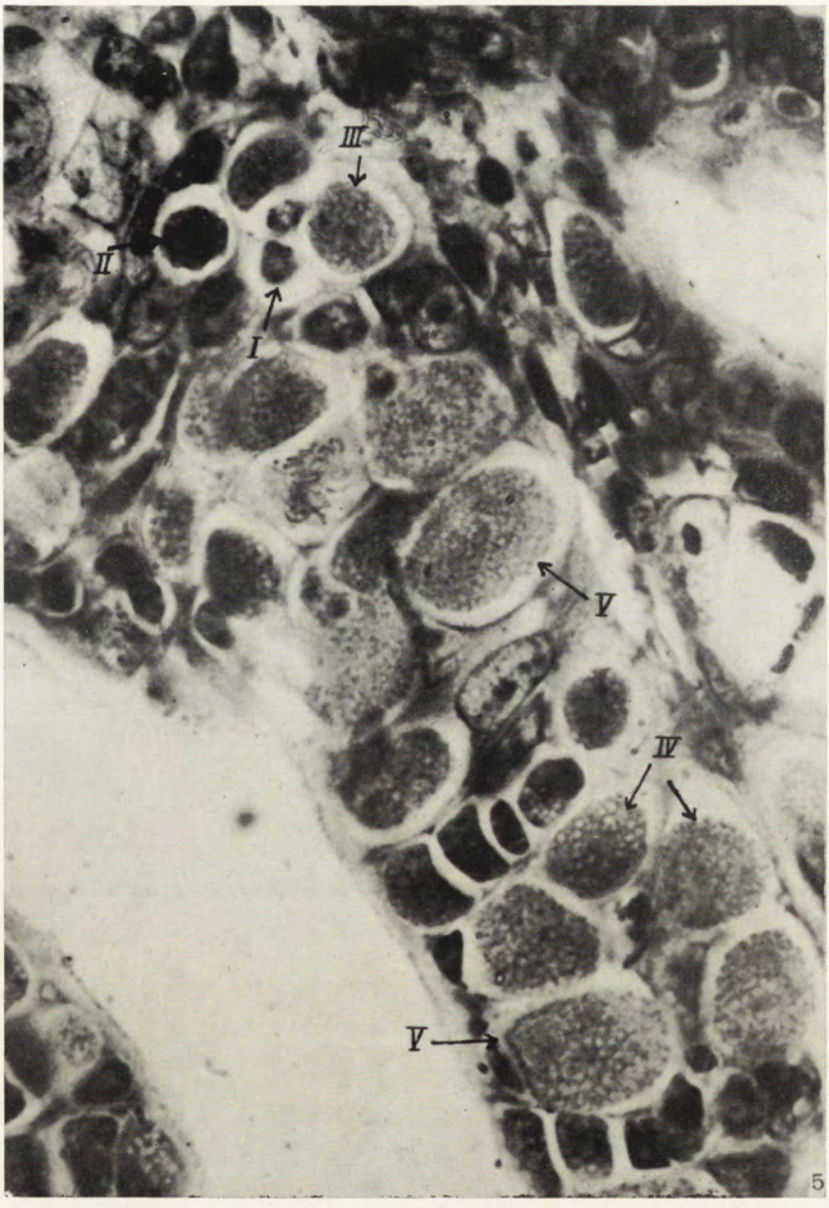

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DNA content of the nuclei and nature of macronuclear chromatin strands of the ciliate *Nassulopsis elegans* (Ehrbg.)

Содержание ДНК в ядрах и природа хроматиновых тяжей макронуклеуса у инфузории *Nassulopsis elegans* (Ehrbg.)

The macronuclei of the majority of ciliates are known to be highly polyploid[1] (Piekarski 1941; Sonneborn 1947; Grell 1950 a, 1953 a, 1962; Fauré-Fremiet 1953; Raikov 1957, 1963; Poljansky and Raikov 1960, 1961). The degree of macronuclear polyploidy is usually determined by comparison of the DNA content of the macronucleus with that of the diploid[2] micronucleus (of course, under conditions that the compared nuclei are in a similar phase of DNA replication — either both in the presynthetic phase G_1, or both in the postsynthetic one — G_2).

Recent photometric studies of the DNA content of the macro- and micronuclei revealed, that the degree of polyploidy of the macronucleus is very unlike in different ciliate species (from several dozens up to several thousands of n), and, moreover, may show considerable variation within one species. Data of such kind were summarized in tabular form in our previous paper (Raikov et al. 1963); studies of some other species, not included into this table, were published later (Dysart 1963; Cheissin and Ovchinnikova 1964; Cheissin et al. 1964).

If the very fact of polyploidy of the macronucleus may be presently considered as firmly established, the question about the state of the numerous chromosomes in the macronucleus is far from being clear. In the majority of ciliates, the macronuclear chromosomes can be seen only with difficulties. However, some descriptions of thread-like chromosome structures in macronuclei of different ciliates did appear during the last years (Grell 1950 b, 1952; Müggе 1957; Schwartz 1958; Sato and Saito 1959; Saito 1961; Saito and Sato 1961; Sato 1963; Kaneda 1960; Seshachar 1960, 1963; Ruthmann und Heckmann 1961; Ruthmann 1963).

[1] An exception is formed by certain lower ciliates (*Trachelocercidae*, *Loxodes*, etc.), in which the macronuclei remain diploid (cf. Raikov 1963; Raikov et al. 1963).

[2] In certain species of ciliates the micronucleus may be in its turn not diploid, but heteroploid to various extent (Chen 1940; Cheissin et al. 1964), which is an additional source of error at determination of the degree of macronuclear polyploidy.

The ciliate *Nassula ornata* proved to be one of the most suitable objects for studies of the macronuclear chromosomes. Anastomozing chromatin strands are distinctly seen on sections through the interphase macronucleus of this species. Shortly before macronuclear division, the chromatin strands become separated from each other and longitudinally splitted, thus behaving like endomitotic chromosomes (Raikov 1962).

Approximate counts of the chromatin strands showed that their number surpassed that of the micronuclear chromosomes by 10—20 times. Consequently, if these strands were separate chromosomes, the degree of polyploidy of the macronucleus of *N. ornata* would be 20—40 n. But photometric determination of the DNA contents of the nuclei of *N. ornata* (Raikov et al. 1963) showed the average degree of macronuclear polyploidy to be 230 n. Thus, chromatin strands of the macronucleus cannot be separate chromosomes — they may be either aggregates of several chromosomes, or polytene structures. We failed to determine more exactly the nature of chromatin strands in *N. ornata,* because their counting proved to be very difficult in this species.

To remove this uncertainity, we tried to find another object, more suitable for such work, among the members of the family *Nassulidae.* The ciliate *Nassulopsis elegans* (Ehrbg.) proved to be such an object. It has a macronucleus with chromatin strands very clearly seen in whole mounts. This allowed to count the strands with sufficient accuracy and, having determined the degree of macronuclear polyploidy of the same specimens, to learn what the chromatin strands of the macronucleus of *Nassulidae* really are.

The author wishes to express his gratitude to E. M. Cheissin, L. P. Ovchinnikova and G. V. Selivanova for valuable advices and placing the necessary apparatus at his disposal.

Material and methods

The work was done with a clone; the initial specimens of *Nassulopsis elegans* were isolated in Spring, 1963, from a sample taken by A. V. Jankowski near Leningrad (Mozhajskoe lake).

Morphological descriptions of this species were given by Kahl 1931, Gelei 1954 and Šramek-Hušek 1957, who included it into the genus *Nassula.* Fauré-Fremiet 1959 transferred this species into the genus *Nassulopsis* created by him. The mechanism of feeding of *N. elegans* was described by Dragesco 1962.

The form used in the present study (Fig. 1 A) corresponds well to the published descriptions, according to all systematic characters (body form evoking *Paramecium caudatum,* length about 170 μ, about 50 kineties, 4 to 5 contractile vacuoles in longitudinal row, adoral zone making a full turn around the body, macronucleus elongate).

The ciliates were cultured, like *Nassula ornata,* in test-tubes at 16—20°C in the Peters' mineral medium and fed with blue-green algae *Oscillatoria brevis* which were cultured separately (see: Raikov 1962). The animals were fixed and attached to slides with the Nissenbaum's 1953 sublimate mixture.

The nuclear DNA was measured on Feulgen stained whole mounts by the method of photographic cytophotometry, which was described in more detail

in our previous paper (R a i k o v et al. 1963). The ciliates were photographed with a 90× (n.a. 1.25) oil immersion objective and a 3× eyepiece in monochromatic green light (546 mμ). The mean extinctions of the nuclei (E) were measured by scanning the negatives with a registering microphotometer at 10-fold magnification. The DNA content of a nucleus was calculated according to the formula: $Q = E \cdot S$, where S is the nuclear area. The latter were measured with a planimeter on enlarged drawings (total magnification 4050 ×); the results (in cm^2) were divided by the square of linear magnification, so that true areas of the nuclei are expressed in $cm^2 \cdot 10^{-7}$. Correspondingly, final values of DNA content of the nuclei are given in $X \cdot 10^{-7}$ arbitrary units.

Results

Nuclear cycle

The nuclear apparatus of n o n - d i v i d i n g specimens of *N. elegans* consists of one elongate macronucleus and several micronuclei (Fig. 1 A; Pl. I 1). The micronuclei are spherical and lie usually close to the macronuclear membrane. The dimensions of the micronuclei are variable enough, even within a specimen; small micronuclei are compact and sharply Feulgen positive, while the larger ones contain a more faintly staining chromatin network.

Within the Feulgen stained macronucleus, more than 100 chromatin strands are clearly seen, which are 6 to 7 μ long and about 1.5 μ thick (Pl. I 1). These strands are usually oriented across the macronucleus, which gives to the latter an appearance of a fir cone. The spaces between the strands are Feulgen-negative and contain small nucleoli.

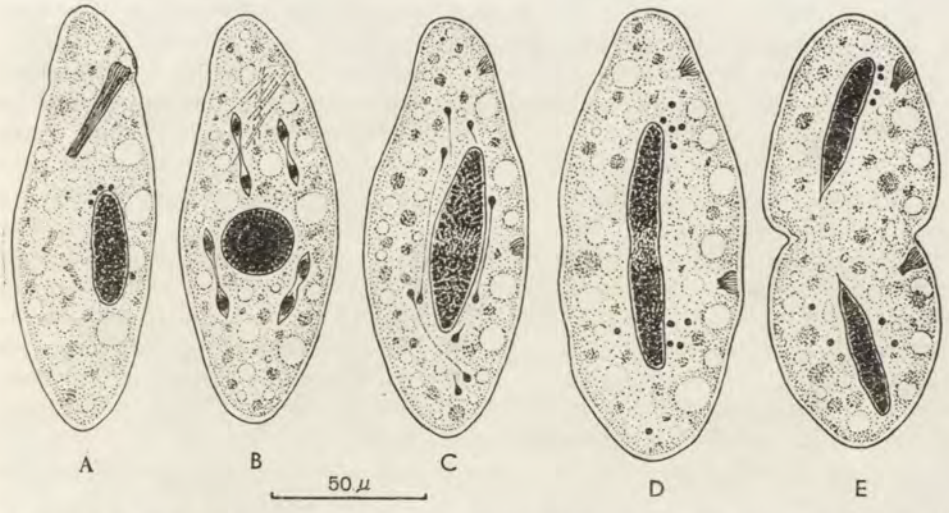

Fig. 1. Nuclear cycle in *N. elegans*. A. Non-dividing specimen. B. Preparation to division ("radial" stage of the macronucleus, anaphase of the micronuclei). C. Early division (stretching of the macronucleus, telophase of the micronuclei). D. Medium stage of division. E. Late division (daughter macro- and micronuclei). Feulgen-Light green, schematized

During preparation to division (Fig. 1 B) the macronuclear shape changes abruptly and becomes spherical. At the same time, the micronuclei divide mitotically. The internal structure of the macronucleus changes as well: the chromatin strands become longer and thinner, they lose their transverse orientation and become interlaced as a tangle of threads (Pl. I 2). At many points, parallel arrangement of two chromatin fibres can be easily seen. This may indicate that a longitudinal splitting of the strands, probably corresponding to endomitosis (endoanaphase stage), took place recently. The micronuclei are at this time in metaphase or early anaphase of mitosis.

At a somewhat later stage of preparation of the macronucleus to division, the daughter chromatin strands shorten and acquire again a regular arrangement (Pl. I 3). Namely, they become oriented exactly along the radii of the spherical macronucleus. The external end of each chromatin strand appears distinctly thickened; moreover, several thickenings are formed along the strand, the dimensions of these thickenings gradually decreasing towards the center of the nucleus. Since homologous thickenings of the neighbouring strands lie at equal distances from the center of the macronucleus, the latter acquires a peculiar concentric structure (Pl. I 3). The number of chromatin strands surpasses at this stage 200 (because two strands are formed after endomitosis from each one of the non-dividing macronucleus). The center of the macronucleus appears filled with a network of faint chromatin fibres. At this stage, the micronuclei are in late anaphase of early telophase (Fig. 1 B).

At the beginning of division the macronucleus stretches, while the micronuclei are in late telophase (Fig. 1 C; Pl. II 4). The chromatin strands lose their radial orientation and become segregated in 2 groups according to the future daughter macronuclei (Pl. II 4). The thickenings along the chromatin strands disappear. Further on, the macronucleus stretches still stronger (Fig. 1 D) and finally divides into daughter nuclei (Fig. 1 E). Only at this time a distinct cytoplasmic constriction appears.

The daughter macronuclei (Fig. 1 E; Pl. II 5) have almost the same internal structure as the non-dividing macronuclei, differing from the latter only by their more elongated shape. The micronuclei are now in early interphase (Fig. 1 D, E; Pl. II 5).

The nuclear events during the division cycle of *Nassulopsis elegans* prove to be closely similar with those of *Nassula ornata* (Raikov 1962). The changes in the chromosomal structures of the macronucleus proceed apparently quite alike in both species, and stages of their nuclear cycle seem to be perfectly homologous. Especially, the stages of preparation of the macronucleus to division — i.e. endomitosis (Pl. I 2) and "radial" (Pl. I 3) stages — are similar up to fine details. As to the differences between the nuclear cycles of *N. elegans* and *N. ornata*, these appear to be restricted to different shapes of the non-dividing macronuclei.

In a closely related species, *Nassulopsis lagenula*, formation of a chromatin extrusion body on the connecting thread of the constricting macronucleus was recently described (Tuffrau 1962). Nuclear reorganization ("parahemixis") was reported to take place in one of the daughter individuals, where the macronucleus becomes replaced at the cost of this extrusion body. In *N. elegans*, however, such phenomena were never observed.

DNA content of the nuclei during the division period

One of the tasks of the present work was determination of the DNA quantities in the macro- and micronuclei just before and immediately after their division. It allowed to reveal the DNA contents of surely postsynthetic (G_2) and surely presynthetic (G_1) nuclei, and to apply these standard values to the analysis of the DNA content of the nuclei of non-dividing ciliates.

Table 1

DNA content of nuclei of *Nassulopsis elegans* during the division period (arbitrary units $\times 10^{-7}$)

Category of individuals	Number of specimens	Macronuclei				Micronuclei			
		number of Ma measured	stage of Ma	DNA content		number of Mi measured	stage of Mi	DNA content	
				extremes	mean			extremes	mean
1. Predivision stages	9	9	endomitosis (G_2)	17.4—18.9	17.7	—	mitosis	not measured	—
2. Early division	4	4	stretching (G_2)	16.8—21.2	18.75	8	after mitosis (G_1)	0.068—0.083	0.074
3. Late division	4	8	daughter Ma (G_1)	8.7—10.3	9.45	11	after mitosis (G_1)	0.064—0.094	0.076
Mean DNA content		G_2 — 18.0 G_1 — 9.45				G_1 — 0.075			
Mean relation of DNA quantities in Ma and Mi in the G_1 phase and the average degree of polyploidy of the Ma								125.5 : 1 (251n)	

The results of the measurements are given in Table 1. The category "Predivision stages" includes individuals preparing to division, with spherical macronuclei (endomitosis and "radial" stages, Fig. 1 B and Pl. I 2, 3). The micronuclei being in mitosis at this stage, we failed to measure their DNA content. The next category, "Early division", is composed of specimens with macronuclei at different stages of stretching, i.e. surely in the G_2 phase (Fig. 1 C, D; Pl. II 4). On the contrary, the micronuclei of these individuals just finished mitosis, i.e. are surely in the G_1 phase. The last category of individuals, "Late division", includes animals with fully divided macronuclei (Fig. 1 E; Pl. II 5). The DNA content was measured separately in each of the two daughter macronuclei and corresponds surely to the presynthetic level (G_1). At this stage, the micronuclei are in the G_1 phase as well.

Table 1 shows that in the phase G_2 (category "Early division") the macronucleus does contains at average 2 times more DNA than in the G_1

phase (18.75 and $9.45 \cdot 10^{-7}$ arbitrary units respectively). During endomitosis (category "Predivision stages"), the mean DNA content of the macronuclei practically equals the postsynthetic level as well ($17.7 \cdot 10^{-7}$ units). Thus DNA synthesis occurs in the macronuclei not during endometaphase-endoanaphase,

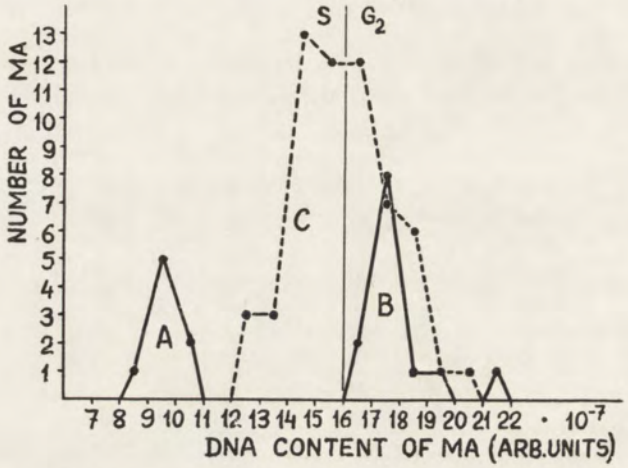

Fig. 2. Frequency distribution of macronuclei according to their DNA content. A — daughter macronuclei in dividing specimens (G_1, n = 8), B — predivision and stretching stages of macronuclei (G_2, n = 13), C — macronuclei of non-dividing animals (S and G_2, n = 58). The vertical line is the arbitrary boundary of the phases S and G_2 in non-dividing macronuclei

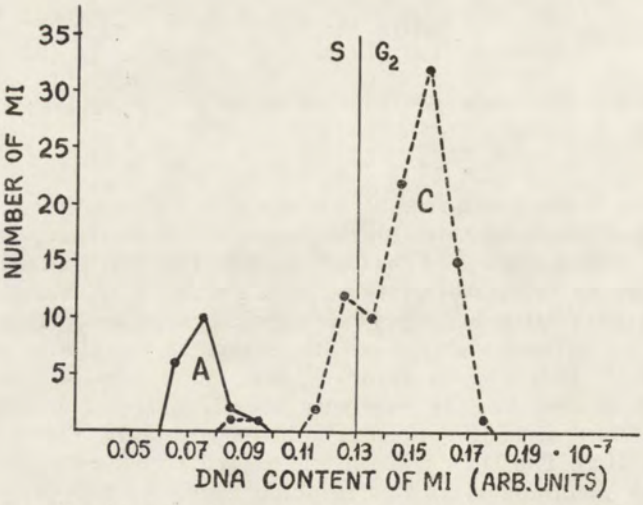

Fig. 3. Frequency distribution of micronuclei according to their DNA content. A — micronuclei immediately after mitosis in dividing animals (G_1, n = 19), C — micronuclei of non-dividing specimens (S and G_2, n = 96). The vertical line is the arbitrary boundary of the S and G_2 phases

and not during the short period between endomitosis and macronuclear division, but at some time during the interval between two cell divisions.

The micronuclei of individuals of the last two categories show practically equal DNA content. At average (calculated for both categories together), the micronucleus contains in the G_1 phase $0.075 \cdot 10^{-7}$ arbitrary DNA units.

The frequency distribution of the measured macronuclei according to their DNA content is given in Fig. 2 (curve A — in the G_1 phase, curve B — in the G_2 phase). An analogous curve for G_1 micronuclei is represented in Fig. 3 (curve A).

DNA content of nuclei of non-dividing specimens

The measurement of nuclear DNA contents in non-dividing *N. elegans* reveals that in a considerable proportion of individuals nuclei (and especially macronuclei) have DNA values intermediate between G_1 and G_2 levels. The frequency distribution according to DNA content of the macronuclei of 58 non-dividing specimens is represented in Fig. 2 (curve C), and that of 96 measured micronuclei of the same specimens — in Fig. 3 (curve C).

In 46 per cent of the non-dividing ciliates the macronuclei contain more than $16 \cdot 10^{-7}$ units (Fig. 2 C). Since similar DNA values are met in the surely postsynthetic macronuclei as well (Fig. 2 B), these 46 per cent of macronuclei will be further considered as having reached the G_2 level. In 54 per cent of specimens, the macronuclei contain from 12 to $16 \cdot 10^{-7}$ DNA units and will be considered as synthesizing ones (phase S). This separation is, of course, quite arbitrary. Neither of the non-dividing specimens studied had macronuclei in the G_1 phase (DNA content 8—$10 \cdot 10^{-7}$ units) or at the beginning of the S phase (10—$12 \cdot 10^{-7}$ units).

The curve of frequency distribution of micronuclei according to their DNA content (Fig. 3 C) shows a clear peak at 0.15—$0.16 \cdot 10^{-7}$ arbitrary units, i.e. at a level twice as high as that of the micronuclei just after mitosis. Thus, the micronuclei of the majority of non-dividing individuals are already in the G_2 phase. There are, however, some micronuclei with transitory DNA values as well, which seem to correspond to the S phase. Their number proves to be about 14 per cent of the total number of micronuclei, if the arbitrary boundary between S and G_2 phases is fixed at $0.13 \cdot 10^{-7}$ DNA units (this figure was chosen by doubling the lowest DNA values of the presynthetic micronuclei of the dividing ciliates).

In many specimens several micronuclei were measured. They contain usually similar DNA amounts. This allows to assume that DNA replication takes place more or less synchronously in several micronuclei of one individual.

Comparing the DNA values of macro- and micronuclei, all non-dividing individuals may be divided into 3 categories (Table 2). Those of the I category (5 specimens only) have both macronuclei and micronuclei[3] in the S phase. In the II category (26 animals) the macronuclei still synthesize DNA, while the micronuclei have already accomplished replication (G_2 phase). In the

[3] Sometimes, during measurement of several micronuclei of one specimen, some of them proved to contain a little more than $0.13 \cdot 10^{-7}$ DNA units, while the other ones — a little less. In these cases the specimen was considered to contain S-phase micronuclei, if the average DNA content of the measured micronuclei of this specimen was less than $0.13 \cdot 10^{-7}$ units.

individuals of the III category (27 specimens) both macro- and micronuclei are in the G_2 phase.

Table 2 shows that the mean DNA content of macronuclei is higher in the II category than in the I, while in the III it is $17.4 \cdot 10^{-7}$ units, almost reaching the average postsynthetic level. In the micronuclei, the mean DNA content in the I category is considerably lower than in the II and the III, where it is practically identical and corresponds to the level expected for the phase G_2 (which is the doubled mean presynthetic level, i.e. $0.150 \cdot 10^{-7}$ units).

Table 2

DNA content of nuclei of non-dividing *Nassulopsis elegans* (arbitrary units · 10^{-7})

Category of individuals	Macronuclei			Micronuclei			Mean relation of DNA quantities of Ma and Mi
	number of Ma	DNA content		number of Mi	DNA content		
		extremes	mean		extremes	mean	
I. Mi S Ma S	5	12.2—15.3	13.9	9	0.088—0.132	0.117	119:1
II. Mi G_2 Ma S	26	12.1—15.9	14.7	53	0.117—0.169	0.148	99:1
III. Mi G_2 Ma G_2	27	16.0—20.5	17.4	34	0.128—0.174	0.149	117:1
Total	58			96			

The analysis of the curves (Figs. 2 and 3) and of Tables 1 and 2 allows to draw some conclusions about the time and the character of DNA replication in *N. elegans* nuclei.

Firstly, the DNA synthesis in macronuclei as well as in micronuclei must begin immediately after plasmotomy (since there are no specimens with presynthetic macro- and micronuclei among the non-dividing animals).

Secondly, the DNA synthesis in the micronuclei must be completed rapidly (since there are few specimens with micronuclei in the S phase). The micronuclei pass the major part of the interdivision interval in the G_2 phase. On the contrary, DNA synthesis in the macronucleus appears to proceed much more gradually and to last during the entire first half of the interdivision interval or even during the first two thirds of it (since more than a half of the non-dividing specimens have macronuclei in the S phase).

Thirdly, in the macronucleus the DNA synthesis seems to begin suddenly and with great intensity, the rate of synthesis gradually slowing down later (since there are many high, few medium and no low intermediate DNA values among the macronuclei in the S phase — see Fig. 2, curve C).

According to these conclusions, a diagram (Fig. 4) was drawn, representing schematically the cycles of DNA replication in the macronuclei and the micronuclei of *N. elegans*. This diagram does not claim for exactitude of the shape of curves and may give only a general idea about the character of the

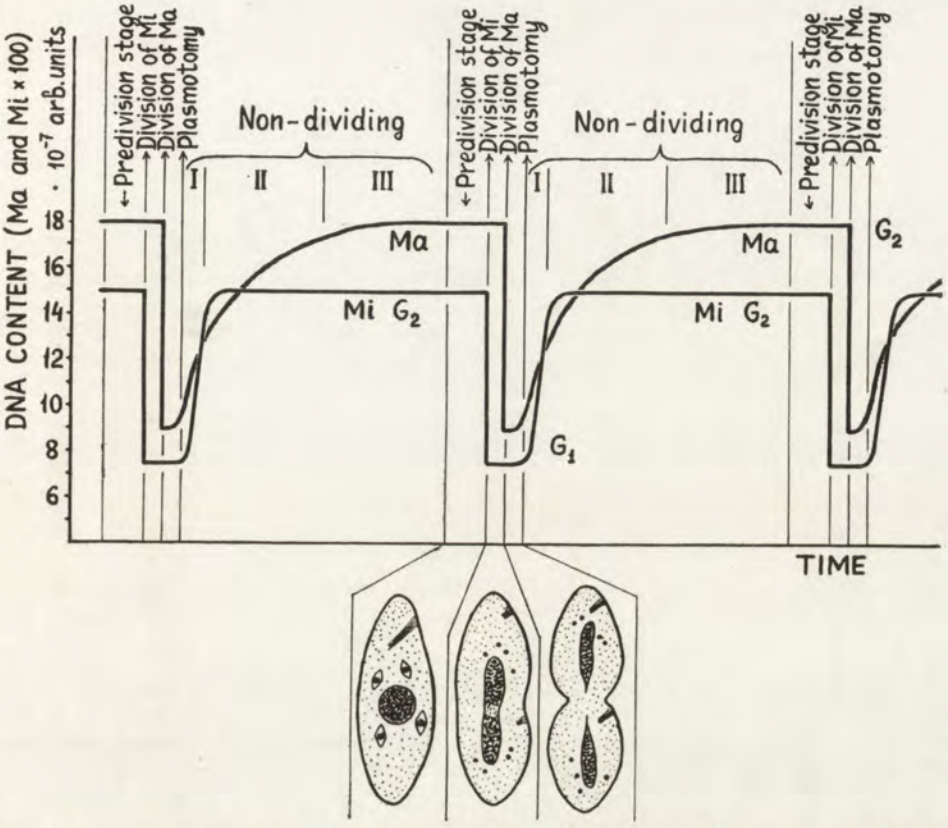

Fig. 4. Diagram of cycles of DNA replication in macro- and micronuclei of. *N. elegans*. The DNA quantity in the micronuclei is multiplied by 100 for convenience in drawing the curves

increase of the nuclear DNA quantity from division to division. On the abscissae axis, the time allotted for each stage was chosen proportionally to the number of specimens of this stage met.

Changes of the nuclear areas

Data about the areas of the macro- and micronuclei during various stages of the nuclear cycle are presented in Table 3. It is to be seen, that the increase of nuclear areas does not coincide in time with DNA replication. Thus, during the S phase the mean area of the macronucleus (and, consequently, its volume) increases but slowly, a sharp growth of this area occurring only at the end of the G_2 phase — i.e. during preparation of the macronucleus to division and especially during its stretching. The S phase micronuclei have practically the same mean area as those in the G_1 phase; an increase of the micronuclear dimensions begins only during the G_2 phase. It is worth to note that during the G_2 phase areas of micronuclei may vary considerably: together with large nuclei ($0.8—0.9 \cdot 10^{-7}$ cm²), small ones are regularly met ($0.3—0.5 \cdot 10^{-7}$ cm², i.e. like G_1 micronuclei).

Table 3

Areas of macronuclei and micronuclei at different stages of the nuclear cycle of *Nassulopsis elegans* ($cm^2 \times 10^{-7}$)

Category of individuals	Macronuclei				Micronuclei			
	phase of the Ma cycle	number of Ma measured	area		phase of the Mi cycle	number of Mi measured	area	
			extremes	mean			extremes	mean
Late division	G_1	8	31.6—42.8	36.6	G_1	11	0.30—0.49	0.41
Non-dividing I	S	5	35.9—43.8	39.0	S	9	0.32—0.55	0.43
Non-dividing II	S	26	30.7—59.8	43.3	G_2	53	0.28—0.92	0.57
Non-dividing III	G_2	27	40.0—60.5	47.2	G_2	34	0.32—0.85	0.59
Predivision stage	G_2	9	42.4—67.0	55.4	mitosis	—	—	—
Early division	G_2	4	70.1—98.6	86.4	G_1	8	0.32—0.50	0.40

Degree of macronuclear polyploidy and number of chromatin strands

The degree of polyploidy of the macronucleus in *N. elegans* may be determined with maximum precision by comparison of the mean quantities of DNA in surely presynthetic macro- and micronuclei (Table 1). The relation of the mean DNA contents of these nuclei is 125.5:1; consequently, if the micronuclei are really diploid, the degree of polyploidy of the macronucleus is at average 251 n. This figure is very close to the theoretically probable value 256 n, which corresponds to 7 successive endomitotic reduplications of chromosomes of the diploid syncaryon.

The degree of macronuclear polyploidy may be also calculated by comparison of the DNA quantities in postsynthetic nuclei (III category of non-dividing animals, Table 2). The relation of the mean quantities of DNA of macro- and micronuclei is there 117:1, thus giving a lower figure of macronuclear polyploidy — 234 n. However, this value seems to be somewhat underestimated, because separation of the macronuclei into S and G_2 groups was done arbitrarily, and the G_2 group could contain some macronuclei with DNA synthesis not completely finished.

The chromatin strands of the macronucleus were counted in 60 non-dividing specimens and in 5 individuals on the "radial" stage of preparation to division (Pl. I 3). The strands were counted thrice in each macronucleus, and the arithmetical mean of the 3 figures was taken as the final result. The individual counts seldomly deviated from the mean more than by 10.

The number of chromatin strands in non-dividing macronuclei varies from 105 to 172, the class 130—140 strands being the modal one (Fig. 5 A). The mean number of strands ($M \pm m$), calculated for 60 non-dividing macronuclei, is 134.4 ± 2.03. As it could be expected, the number of strands after their endomitotic duplication, during preparation of the macronucleus to division, proved to be approximately two times as high — from 246 to 275, at average — 258 strands (Fig. 5 B).

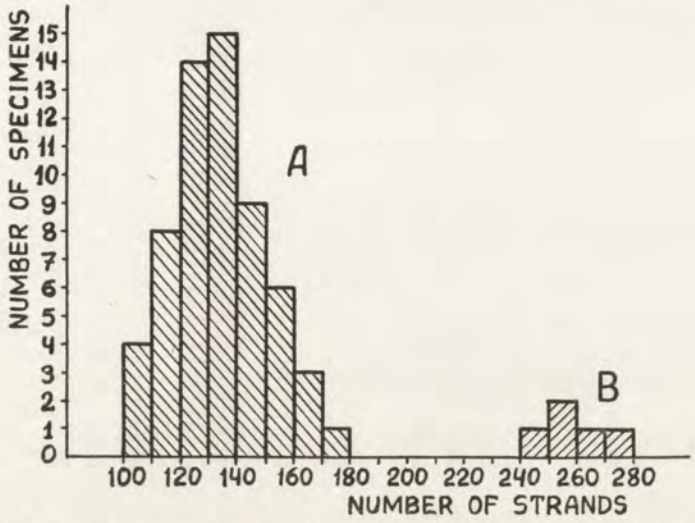

Fig. 5. Frequency distribution of *N. elegans* according to the number of chromatin strands in the macronucleus. A — non-dividing specimens (n = 60), B — predivision stages, after endomitosis (n = 5)

Thus, the mean number of chromatin strands of the non-dividing macronucleus (134) lies very close to the figure determining the relation of DNA quantity in the macronucleus to that in the micronucleus (125.5 : 1). Correspondingly, the number of strands is nearly two times less than the degree of macronuclear polyploidy (251 n). A conclusion may be drawn from it, that the chromatin strands of the macronucleus of *N. elegans* are diploid aggregates of chromosomes.

Discussion

The degree of polyploidy of the macronucleus of *Nassulopsis elegans* (251 n) proved to be close enough to that of *Nassula ornata* (about 230 n). In both cases 7 endomitotic cycles are necessary for development of the macronucleus from a diploid syncaryon derivate. This conformity holds in spite of the fact that the absolute DNA content is about 4—5 times higher in the macronucleus and the micronuclei of *N. ornata* (Raikov et al. 1963), than in the respective nuclei of *N. elegans*[4]. It would be very interesting to study photometrically some other representatives of *Nassulidae*: it may occur that the number of endomitotic cycles is firmly fixed in this taxonomic group. This would be the more interesting, since in other ciliate groups the degree of macronuclear polyploidy is highly variable in different species of a genus (*Paramecium* — cf. Cheissin and Ovchinnikova 1964) as well as

[4] Although in both cases the results were expressed in arbitrary DNA units and although fixation and staining was done at different times, the values of DNA content of nuclei of *Nassula ornata* and *Nassulopsis elegans* seem to be more or less comparable, because both investigations were done with the same apparatus and methods.

within a species (*Tetrahymena limacis, T. patula* and *T. rostrata* — Dysart 1963).

The replication of DNA takes place in *Nassulopsis elegans* during early interphase in both types of nuclei. Other ciliates show considerable diversity in this respect. The macronuclei of *Paramecium caudatum* and *P. aurelia* synthesize DNA during the second half of the interphase (Walker and Mitchison 1957; Cheissin et al. 1963; Kimball and Barka 1959; Woodard et al. 1961), while macronuclei of *Paramecium trichium* do during the first half of it (Hanson and Twichell 1962). Within the species *Tetrahymena pyriformis,* some strains synthesize macronuclear DNA at the beginning of the interdivision interval (Prescott 1960), while others — in the middle of it (McDonald 1958, 1962), and still others — at the end of this interval (Walker and Mitchison 1957). It is very characteristic that the synthesis of DNA takes always much more time in macronuclei than in micronuclei; in some cases it lasts nearly all the interphase (*Stentor* — Guttes and Guttes 1960; *Euplotes* — Prescott et al. 1962). This fact may be attributed to asynchrony of DNA replication in separate genomes of the polyploid macronucleus, which is most clearly evident in *Euplotes,* where DNA replication proved to be connected with reorganization bands moving through the macronucleus (Gall 1959).

In micronuclei, the time of DNA replication varies strongly as well. In *Chilodonella uncinata* (Seshachar 1950) and *Tetrahymena pyriformis* (McDonald 1962) the micronuclear DNA replicates just after mitosis, in *Euplotes eurystomus* — even during anaphase and telophase (Prescott et al. 1962), while in *Paramecium aurelia* it does at the beginning of the second half of the interphase (Woodard et al. 1961) and in *P. caudatum* — at the end of the interphase (Walker and Mitchison 1957). The time of DNA syntheses coincides in macro- and micronuclei by far not always: while in *Nassulopsis elegans, Paramecium caudatum* and *P. aurelia* both nuclei begin DNA replication simultaneously, in *Tetrahymena pyriformis* and *Euplotes eurystomus* macro- and micronuclei synthesize DNA at different periods of the division cycle.

Theoretically important is the question about the DNA content of the macronucleus during the morphologically visible endomitosis (i.e. endometaphase-endoanaphase). It may be expected that DNA replication should occur during endointerphase, long before visible endomitosis (like in mitotic cycles of the majority of cells). According to Zybina 1963, this is exactly the case in endomitotic mammalian cells. The above presented data on *Nassulopsis elegans* show that the ciliate macronucleus is in this respect similar: DNA replication terminates in this nucleus long before morphologically visible endomitosis. During endometaphase-endoanaphase, the macronucleus is already in the G_2 phase.

If this is true for macronuclei of all ciliates, then the common statement, that reorganization bands in *Euplotes* are "waves of endomitoses", appears to be inexact. Reorganization bands are in reality waves of replication of DNA molecules, and endometaphase-endoanaphase stages must be probably looked for during later stages of the nuclear cycle (perhaps during macronuclear condensation).

Finally, the question about the state of chromosomes within the macronucleus deserves attention. If the macronucleus contained a multitude of

separate chromosomes, their random segregation during "amitotic" division of this nucleus would lead to a rapid increase of aneuploidy. This is apparently not the case, since the macronuclear genotype remains usually unaltered in clones, and many ciliate species may multiply for a long time without periodical replacement of the macronucleus. These considerations led Sonneborn 1947 and Grell 1953a, 1962 to the opinion that chromosome complexes (genomes) are more or less separated from each other within the macronucleus and distributed as whole units between the daughter nuclei during macronuclear division. Thus, the "amitosis" of the macronucleus could be more correctly called segregation of genomes.

Sonneborn believes that the macronucleus may consist of many diploid subnuclei, the latter being capable to divide within the macronucleus. However, no morphological evidence supporting this view could be obtained until now.

Meanwhile, the chromatin strands in the macronucleus of *Nassulopsis* (and probably also in that of *Nassula*) correspond almost exactly to Sonneborn's notion of subnuclei. They are diploid chromosome complexes, which become segregation units during macronuclear division. Before division of the macronucleus, they undergo autonomous duplication, the number of diploid subnuclei being twice as high just before division than in non-dividing macronuclei.

Grell's opinion differs somewhat from Sonneborn's view. Grell believes that the intragenomic union of chromosomes may be brought about by end-to-end junction of chromosomes resulting in formation of a chain called "Sammelchromosome" (complex or compound chromosome). Each "Sammelchromosome" may correspond to a haploid genome. Such phenomena were really observed by Grell 1953b in highly polyploid nuclei of the radiolarian *Aulacantha*; recently, similar structures were found also in the macronucleus of the ciliate *Loxophyllum* (Ruthmann 1963).

The advantage of Grell's view is that it can explain the mechanism of endomitotic reduplication of the macronuclear chromosomes. A "Sammelchromosome" may split longitudinally as a whole, without disintegration into its components (single chromosomes), no mitotic spindle being necessary for this process. If it is the case, the daughter "Sammelchromosomen" would be indentical to each other and to the "parent" genome.

The data on the macronuclei of *Nassulopsis* (and *Nassula*) are by no means contradictory with Grell's views as well. At the endomitosis stage (Pl. I 2), the chromatin strands stretch into long fibres, which split longitudinally. It is possible to suggest that these fibres are nothing else than "Sammelchromosomen", though no boundaries of single chromosomes are visible in them. In the chromatin strands of non-dividing macronuclei the "Sammelchromosomen" may be twisted so that they become invisible.

However, each chromatin strand of *Nassulopsis* contains not one, but two haploid genomes; consequently, according to Grell, two "Sammelchromosomen" should be included therein. It is not yet clear, why they are not seen separately from each other during endomitosis. Perhaps, they lie close together (something like somatic conjugation of chromosomes), as it was described in developing anlagen of *Nyctotherus* macronuclei (Golikova 1964).

According to our opinion, there are no serious discrepancies between Sonneborn's theory of subnuclei and Grell's theory of "Sammelchromosomen". In any case, the chromatin strands of the macronucleus of *Nassulopsis* may be interpreted as diploid subnuclei as well as pairs of "Sammelchromosomen". Assuming that Sonneborn's subnuclei do not multiply by mitosis (as he suggested only in his early papers), but contain pairs of "Sammelchromosomen" and replicate by endomitosis, both hypotheses may be brought together. But doing it, we should not forget that macronuclei of the majority of ciliates do not contain such peculiar chromatin strands, as macronuclei of *Nassulidae*. In these forms the "Sammelchromosomen" may not unite pairwise into subnuclei. Thus, formation of "Sammelchromosomen" might be a general rule for all polyploid macronuclei, while their union in diploid subnuclei might be a more special case.

Summary

The macronucleus of *Nassulopsis elegans* (Ehrbg.) contains at average about 134 chromatin strands, splitting longitudinally by endomitosis before macronuclear division. During division of the macronucleus, the strands are segregated between the daughter nuclei.

The DNA replication in the macronucleus of *N. elegans* begins immediately after plasmotomy and lasts during the whole first half of the interdivision interval. During preparation of the macronucleus to division (endomitosis stage), this nucleus is already in the G_2 phase.

In the micronuclei, the DNA synthesis begins soon after mitosis (simultaneously with the beginning of the macronuclear synthesis), goes on rapidly and ends much earlier than in the macronucleus. The majority of non-dividing specimens have micronuclei in the G_2 phase.

The increase of the dimensions of the macro- and micronuclei does not coincide in time with DNA synthesis in these nuclei, but takes place later.

The average degree of polyploidy of the macronucleus of *N. elegans*, calculated by comparison of the DNA contents of presynthetic (G_1) nuclei, is 251 n. Since the number of chromatin strands in the macronucleus is nearly two times smaller than this figure, the strands should be considered as diploid chromosome aggregates (subnuclei). The question about the structure of the macronucleus of the ciliates is discussed (Sonneborn's theory of subnuclei and Grell's theory of "Sammelchromosomen").

РЕЗЮМЕ

Макронуклеус *Nassulopsis elegans* (Ehrbg.) содержит в среднем около 134 хроматиновых тяжей, расщепляющихся продольно (эндомитоз) перед делением макронуклеуса. Во время деления макронуклеуса тяжи распределяются между дочерними ядрами.

Количество ДНК в ядрах *N. elegans* определялось путем фотометрирования препаратов, окрашенных по Фельгену. Редупликация ДНК в макронуклеусе начинается сразу после плазмотомии и заканчивается несколько позже середины интерфазы. Микронуклеусы синтезируют ДНК вскоре после митоза и большую часть времени проводят в фазе G_2. Увеличение размеров макро- и микронуклеусов не совпадает по времени с синтезом ДНК, а наступает позже.

Средняя степень полиплоидности макронуклеуса *N. elegans*, вычисленная путем сравнения количеств ДНК в пресинтетических ядрах, равна 251 n. Поскольку число хроматиновых тяжей в макронуклеусе почти в 2 раза меньше этой цифры, тяжи должны считаться диплоидными агрегатами хромосом (субнуклеусами). В связи с этим обсуждается вопрос о структуре макронуклеуса инфузорий (теория субнуклеусов Соннеборна и теория сборных хромосом Грелля).

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EXPLANATION OF PLATES I—II

Photomicrographs of whole Feulgen stained mounts of *N. elegans* (×2160)

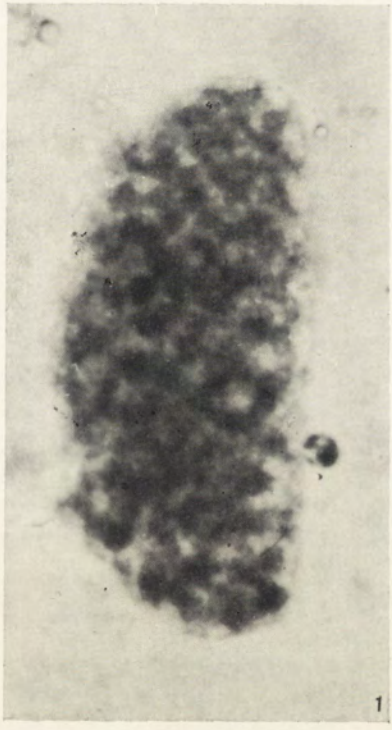
1: The macronucleus and one of the micronuclei of a non-dividing specimen.

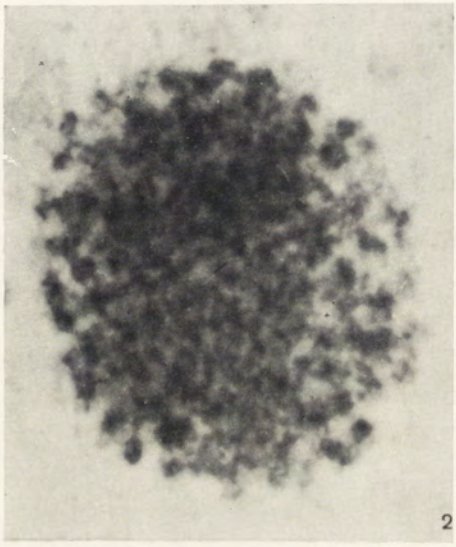
2: Endomitosis (endoanaphase stage) during preparation of the macronucleus to division.

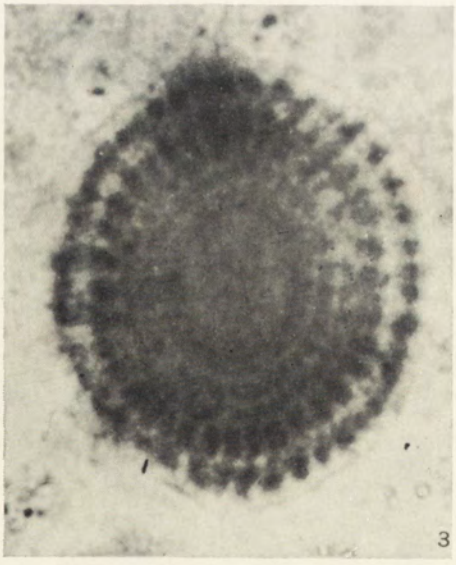
3: Preparation of the macronucleus to division: radial orientation of the daughter chromatin strands.

4: Beginning of the macronuclear stretching; the micronuclei are in late telophase.

5: One of the two daughter macronuclei of a dividing animal; 2 micronuclei are seen.

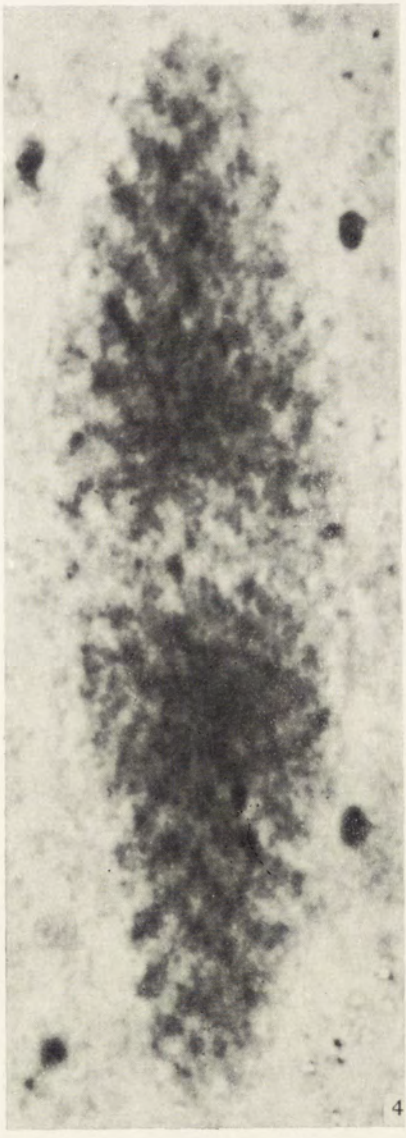


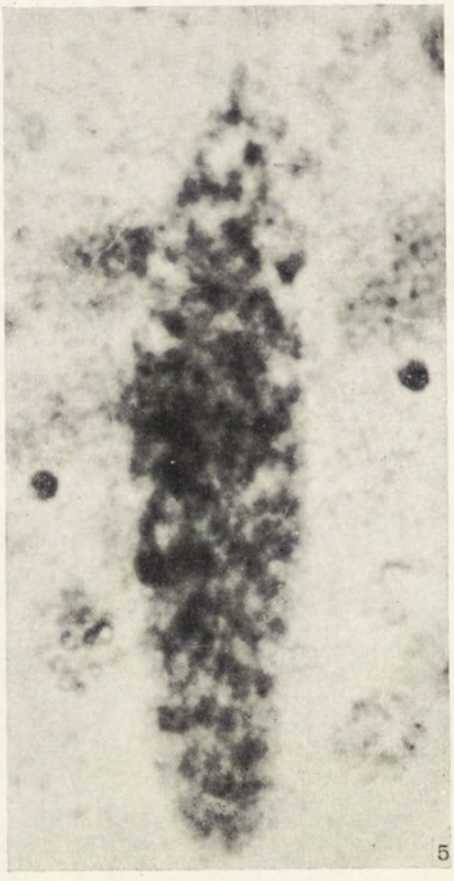




10 μ

I. B. Raikov auctor phot.





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I. B. Raikov auctor phot.

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Жизненный цикл *Trichodina cottidarum* Dogiel, 1948 (*Peritricha, Urceolariidae*); фотометрическое изучение динамики ДНК в макронуклеусе

The life cycle of *Trichodina cottidarum* Dogiel, 1948 (*Peritricha, Urceolariidae*); a photometric study of DNA changes in the macronucleus

Изучение *Trichodina cottidarum* с жабр морских рыб показало, что в зависимости от стадий жизненного цикла происходят изменения морфологии ядерного аппарата, сопровождающиеся изменениями в распределении ДНК. В связи с этим возникла необходимость количественного анализа динамики ДНК, что и было выполнено с помощью цитофотометрирования фотографическим методом.

Мы приносим свою искреннюю благодарность заведующему лаборатории микроскопии Института Цитологии АН СССР профессору Е. М. Хейсину, сотрудникам лаборатории Л. П. Овчинниковой и Г. В. Селивановой за постоянные консультации при освоении метода, а также лаборантам лаборатории цитологии одноклеточных организмов Н. А. Махновской и М. А. Френкель за участие в обработке материалов.

Материал и методика

Исследовали сборы по *T. cottidarum* (ff. *maris-albi, barenzi, cyclopteri*) из Белого и Баренцова морей. Данные по динамике ДНК в жизненном цикле получены главным образом для *T. cottidarum* f. *maris-albi* с жабр бычка *Myoxocephalus scorpius* (Белое море). Тотальные препараты триходин, зафиксированные на мазках жидкостью Шаудинна и окрашенные по Фельгену (гидролиз 5 мин в 1 н HCl при 60°C), фотографировали с помощью микроскопа МУФ-6 в монохроматическом зеленом свете (линия 546 mμ) при окуляре 3× и объективе 90×. Площади ядер измеряли планиметром по рисункам, сделанным с негативов через фотоувеличитель при 15-кратном увеличении. Негативы фотометрировали на сканирующем микрофотометре МФ-4 при 10-кратном увеличении. Площадь ядра выражали в см² (×10⁻⁷), количество ДНК в условных единицах (× 10⁻⁷). В дальнейшем изложении сомножитель × 10⁻⁷ опускается. Промерено 370 экземпляров инфузорий. Полученные результаты обработаны вариацион-

но-статистически. Подробное описание методики приводится в работе Райкова, Хейсина и Бузе (Raikov et al. 1963).

Большинство работ, появившихся в последние годы и посвященных количественному исследованию ДНК в ядрах многих инфузорий, в том числе и кругоресничных, были сделаны на свободноживущих инфузориях и преимущественно на клональном материале. Имея дело со смешанными природными популяциями паразитических инфузорий с рыб разного возраста и физиологического состояния, мы столкнулись с большой неоднородностью материала, что очень затруднило его обработку.

Результаты

Содержание ДНК в макронуклеусах вегетативных и делящихся инфузорий

Дочерние макронуклеусы (Ма) только что разделившихся или еще не закончивших плазмотомию *T. cottidarum* f. *maris-albi* (Табл. I 1) содержат наименьшее для вегетативных особей количество ДНК (в среднем 9.1 усл. ед. — Таблица 1). У разных экземпляров инфузорий количество ДНК варьирует от 7.2 до 12.2 усл. ед., то есть крайние величины различаются почти вдвое. По мере роста Ма количество ДНК увеличивается и становится максимальным перед началом очередного деления (в среднем 22.7, при индивидуальных вариациях в пределах от 16.1 до 26.8 усл. ед. — Таблица 1). Сопоставляя величины, полученные при измерении ДНК в начале и в конце интерфазного периода, можно говорить об удвоении ДНК к концу интерфазы, хотя из-за большой индивидуальной изменчивости соотношение 1 : 3 соблюдено не очень точно. По-видимому, Ма недавно разделившихся инфузорий по количеству ДНК можно отнести к пресинтетическим, а ядра инфузорий, приступающих к новому делению, к постсинтетическим. Ядра вегетативных особей, обладающие характерной подковообразной формой (Табл. I 2—4), занимают промежуточное положение. Содержание ДНК в них в среднем равно 13.5 при индивидуальной изменчивости от 6.4 до 19.7 усл. ед. (Таблица 1).

Несколько иные результаты, по сравнению с *T. cottidarum* f. *maris-albi*, были получены для *T. cottidarum* f. *barenzi*. У этих инфузорий после деления также происходит увеличение содержания ДНК в Ма, но, видимо, синтез заканчивается несколько раньше, чем в предыдущем случае. Максимальное (удвоенное) количество ДНК наблюдается уже в Ма вегетативных особей, которые не обнаруживают признаков деления (17.0, по сравнению с 8.5 усл. ед. у только что разделившихся — Таблица 1). Несколько неожиданным оказалось количество ДНК в Ма *T. cottidarum* f. *barenzi* перед началом деления (13.7, по сравнению с 17.0 усл. ед. в подковообразных Ма). Вероятнее всего, что этот результат в какой-то мере является случайным из-за того, что выборка оказалась очень мала (6 экз. — Таблица 1). Как и у *T. cottidarum* f. *maris-albi*, вегетативные Ма *T. cottidarum* f. *barenzi* по количеству ДНК можно разделить на пре- и постсинтетические (в среднем 8.5 и соответственно 17.0 усл. ед.). Несомненно, что такое деление ядер на пре- и постсинтетические в значительной степени является условным, так как обе группы частично перекрывают друг друга.

При изучении динамики ДНК в ядре большой интерес представляет вопрос о том, когда именно происходит синтез. В работах разных исследователей было показано, что в ядрах инфузорий, так же как и в ядрах многих клеток многоклеточных, синтез ДНК происходит в течение интерфазы. В Ма *Paramecium*

Таблица 1

Содержание ДНК и площадь Ma *Trichodina cottidarum* Dogiel, 1948 на разных стадиях жизненного цикла

Content of DNA and area of Ma in *Trichodina cottidarum* Dogiel, 1948 on different stages of their life cycle

Стадия Stage	*T. cottidarum* f. *maris-albi*						*T. cottidarum* f. *barenzi*					
	площадь в $см^2 \times 10^{-7}$ area in $cm^2 \times 10^{-7}$			количество ДНК в усл. ед. $\times 10^{-7}$ content of DNA, arb. un. $\times 10^{-7}$			площадь в $см^2 \times 10^{-7}$ area in $cm^2 \times 10^{-7}$			количество ДНК в усл. ед. $\times 10^{-7}$ content of DNA, arb. un. $\times 10^{-7}$		
	число экз. number	крайние значения extremes	M±m mean	число экз. number	крайние значения extremes	M±m mean	число экз. number	крайние значения extremes	M±m mean	число экз. number	крайние значения extremes	M±m mean
вегетативные особи vegetative individuals	32	20.3—57.4	39.1±1.3	32	6.4—19.7	13.5±0.7	40	20.6—40.0	28.1±0.6	52	8.9—28.4	17.0±0.6
вегетативн. особи перед делен. vegetat. indiv. before div.	6	25.4—58.2	34.3±5.3	6	16.1—26.8	22.7±1.6	6	10.1—23.8	15.8±1.8	6	9.2—20.9	13.7±1.8
вегетативн. особи после делен. vegetat. indiv. after div.	7	18.4—28.3	23.8±1.5	7	7.2—12.2	9.1±0.8	8	8.2—15.9	12.0±1.5	8	2.7—13.7	8.5±1.2
конъюганты conjugants												
до первого метагамн. деления before Ist metagam. div.	14	21.8—49.2	35.7±2.6	14	5.7—19.7	11.1±0.9	—	—	—	—	—	—
после первого метагамн. делен. after Ist metagam. div.	12	0.8—1.6	1.1±0.1	12	0.1—0.4	0.2±0.03	61	0.8—7.8	3.8±0.2	61	0.2—1.1	0.6±0.03
,, ,, ,,	24	5.0—18.0	8.8±0.9	24	0.7—4.7	2.4±0.2	18	1.3—9.3	4.4±0.9	18	0.1—2.6	0.8±0.2
после второго метагамн. делен. after 2nd metagam. div.	—	—	—	—	—	—	31	1.2—16.3	6.3±0.7	31	0.3—11.3	3.2±0.6
после третьего метагамн. делен. after 3 rd metagam. div.	6	18.0—30.0	22.0±2.0	6	4.3—10.3	6.7±1.1	—	—	—	—	—	—
вегетативные особи vegetative individuals	—	—	—	—	—	—	13	12.0—32.7	19.2±2.0	13	10.1—24.6	15.6±0.5
	32	20.3—57.4	39.1±1.3	32	6.4—19.7	13.5±0.7	40	20.6—40.0	28.1±0.6	52	8.9—28.4	17.0±0.6

caudatum он имеет место во второй половине интерфазы и удвоение достигается только перед самым концом плазмотомии (Хейсин и др. 1963). По данным Уокера и Митчисона (Walker and Mitchison 1957) и Мак Дональд (McDonald 1962) у *Tetrahymena pyriformis* и *Paramecium caudatum* синтез ДНК осуществляется в течение всей интерфазы, причем наиболее интенсивно в конце этого периода. Что касается *T. cottidarum*, то у них синтез также происходит в интерфазе и, по-видимому, в течение всего периода. Количество ДНК становится максимальным в конце интерфазы перед самым делением (*T. cottidarum* f. *maris-albi*) или несколько раньше (*T. cottidarum* f. *barenzi* — Таблица 1). Предположение о том, что деление микронуклеуса (Ми) является толчком для начала синтеза ДНК, высказанное Хейсиным и др. 1963 в отношении *P. caudatum*, по всей видимости, не применимо к триходинам, так как максимальное количество ДНК в Ма наблюдается задолго до начала деления Ми.

Содержание ДНК в Ма конъюгантов

Половой процесс у триходин начинается с того, что одна из инфузорий своим задним концом (розеткой) прикрепляется к ротовому концу партнера. При этом ни морфологически (Табл. II 5), ни по количеству ДНК в Ма конъюганты существенно не отличаются от нормальных вегетативных особей (Таблица 1). Можно предположить, что в ядрах будущих конъюгантов после деления замедляется или вообще прекращается синтез ДНК. Это предположение кажется весьма вероятным в свете данных Мэзия (Mazia 1961) о том, что в клетках, которым не предстоит деление, количество ДНК не увеличивается.

Содержание ДНК в Ма эксконъюгантов

В результате деления синкариона в каждом из эксконъюгантов возникают 7 зачатков будущих Ма. На ранних стадиях, когда зачатки еще слабо красятся по Фельгену (Табл. II 6), количество ДНК в каждом из них варьирует от 0.1 до 0.4 усл. ед. у *T. cottidarum* f. *maris-albi* и от 0.2 до 1.1 усл. ед. у *T. cottidarum* f. *barenzi* (Таблица 1). Поскольку зачатки являются продуктами деления диплоидного синкариона, интересно было сравнить содержание ДНК в них и в Ми, которые, вероятно, также являются диплоидными. Оказалось, что количество ДНК в зачатках Ма на стадии, предшествующей первому метагамному делению, уже в 15 раз превышает среднее (0.04 усл. ед.) и в 5 раз максимальное (0.12 усл. ед.) количество ДНК в Ми вегетативных особей. На основании этих данных можно судить о том, что синтез ДНК в зачатках Ма начинается очень рано. Различия в количестве ДНК у разных зачатков одного эксконъюганта, вероятно, можно объяснить неодинаковой скоростью синтеза ДНК в них.

В ходе трех последующих метагамных делений зачатки распределяются между дочерними особями (Табл. II 7—8), так что каждая инфузория получает по одному будущему Ма. При этом происходит рост зачатков, сопровождающийся увеличением в них содержания ДНК (Таблица 1). В результате после третьего метагамного деления единственный зачаток содержит примерно столько же ДНК (15.6 усл. ед.), сколько и взрослое вегетативное ядро (17.0 усл. ед.). Судя по полученным данным, характер синтеза ДНК в зачатках Ма у *T. cottidarum* f. *maris-albi* и f. *barenzi* несколько различен: по-видимому, если основываться на средних величинах, у беломорских триходин наиболее энергично синтез протекает в первой (до второго метагамного деления), а у баренцовоморских во второй половине периода реорганизации. Аналогичный процесс постепенного накопления ДНК и увеличения объёма зачатков по мере их развития был опи-

сан Сешахаром и Дасс (Seshachar and Dass 1954 a) для свободноживущих кругоресничных инфузорий *Epistylis articulata*. Подобно тому, что мы наблюдали у *T. cottidarum* f. *maris-albi*, у *E. articulata* происходит интенсивный синтез ДНК на начальных стадиях, а затем скорость его замедляется и процесс идет более равномерно. Такой же рост содержания ДНК параллельно увеличению зачатков ДНК происходит у *E. articulata* при регенерации Ма из вегетативного Ми (Seshachar and Dass 1954 b).

Обсуждение результатов

Как видно из Таблицы 2, среднее количество ДНК в Ма вегетативных особей не является характерной особенностью той или иной формы *T. cottidarum* и не зависит от хозяина и места обитания (Белое и Баренцово море). Отсутствует единообразие и внутри одной формы. Так статистически достоверными ($p < 0.1\%$) являются различия между *T. cottidarum* f. *barenzi* с разных хозяев — с *Myoxocephalus* и *Gymnacanthus* — в Баренцовом море между *T. cottidarum* f. *maris-albi* с трех экземпляров *Myoxocephalus* в Белом море. В то же время одна из популяций *T. cottidarum* f. *maris-albi* из Белого моря мало отличается от *T. cottidarum* f. *barenzi* с *Myoxocephalus* из Баренцова моря, а *T. cottidarum* f. *barenzi* с *Gymnacanthus* почти не отличается от *T. cottidarum* f. *cyclopteri* с *Cyclopterus* ($p > 5\%$). Достоверности различий количества ДНК в Ма разных форм и популяций приведены в Таблице 3.

Чем объяснить столь значительные различия в содержании ДНК в Ма инфузорий, принадлежащих к одному и тому же виду? По всей вероятности причина не только в том, что мы имели дело с естественными популяциями. Значительную изменчивость неоднократно отмечали при изучении клонального материала у разных видов *Tetrahymena* (Walker and Mitchison 1957, Dysart 1962, 1963), *Paramecium* (Walker and Mitchison 1957, Blanc 1963; Raikov et al. 1963, Cheissin et al. 1964). Вероятно такую изменчивость у триходин можно связать с различным содержанием ДНК в Ма разных линий (в пределах естественной популяции), неравномерным распределением ядерного вещества при делении Ма, а также с разной скоростью синтеза в отдельных зачатках и взрослых Ма.

Сравнивая содержание ДНК в Ма триходин разных размеров, мы приходим к выводу, что в общем вегетативные Ма более крупных форм, по сравнению с мелкими, имеют больше ДНК (Таблица 2). Однако, эта зависимость не является строгой и постоянной. Примером могут служить три популяции беломорских триходин, в которых при одинаковых размерах Ма инфузории достоверно различаются по содержанию ДНК ($p < 0.1\%$ — Таблица 3).

Не имея данных по числу хромосом в Ми и испытывая затруднения при разделении Ми по содержанию ДНК на пре- и постсинтетические, мы не решились в этой работе обсуждает вопрос о степени полиплоидности Ма триходин, определив лишь соотношение ДНК в Ма и Ми у разных форм и популяций *T. cottidarum*. Из Таблицы 2 видно, что самое маленькое соотношение (242 : 1) было в третьей популяции *T. cottidarum* f. *maris-albi*, а самое большое (758 : 1) у *T. cottidarum* f. *cyclopteri*. В остальных случаях соотношения были 290 : 1, 376 : 1, и 426 : 1. По данным Cheissin et al. 1964, у *P. caudatum* содержание ДНК в Ма и Ми находится в определенном соотношении. При наличии мелких Ми с минимальным содержанием в них ДНК всегда наблюдается сравнительно не-

Таблица 2

Сравнение разных форм и популяций *Trichodina cottidarum* Dogiel, 1948 из Белого и Баренцова морей

Comparison of different forms and populations of *Trichodina cottidarum* Dogiel, 1948 from the White Sea and the Barents Sea

Море Sea	Форма Form	Хозяин Host	Площадь Ma в $см^2 \times 10^{-7}$ Ma area in $cm^2 \times 10^{-7}$			Количество ДНК в Ma в усл. ед. DNA content in arb. un. in Ma			Количество ДНК в Ми в усл. ед. DNA content in arb. un. in Mi			Соотношение количества ДНК в Ma и Ми Ratio of DNA contents in Ma and Mi
			число экз. number	крайние значения extremes	M±m mean	число экз. number	крайние значения extremes	M±m mean	число экз. number	крайние значения extremes	M±m mean	
Белое White	*maris-albi*	*Myoxocephalus* (I)	21	24.7—53.0	38.9±1.8	38	7.2—47.0	18.8±1.4	21	0.01—0.14	0.05±0.01	376:1
Белое White	*maris-albi*	*Myoxocephalus* (II)	32	20.3—57.4	39.1±1.3	32	6.4—19.7	13.5±0.7	22	0.01—0.10	0.05±0.01	290:1
Белое White	*maris-albi*	*Myoxocephalus* (III)	22	28.2—49.2	39.1±1.2	30	4.4—16.6	9.7±0.6	21	0.01—0.10	0.04±0.01	242:1
Баренцово Barents	*barenzi*	*Myoxocephalus*	40	20.6—40.0	28.1±0.6	52	8.9—28.4	17.0±0.6	40	0.01—0.12	0.05±0.004	426:1
Баренцово Barents	*barenzi*	*Gymnacanthus*	25	26.3—61.0	48.5±2.1	25	14.9—47.0	29.5±1.7	—	—	—	—
Баренцово Barents	*cyclopteri*	*Cyclopterus*	30	27.9—169.0	73.7±8,3	29	6.3—81.1	30.3±3.9	10	0.01—0.10	0.04±0.01	758:1

Таблица 3

Достоверность различий количества ДНК в Ма разных форм и популяций *T. cottidarum*
Significance of differences of the DNA content in different forms and populations of *T. cottidarum*

	maris-albi Myoxocephalus (I) M±m = 18.8±1.4	*maris-albi Myoxocephalus* (II) M±m = 13.5±0.7	*maris-albi Myoxocephalus* (III) M±m = 9.7±0.6	*barenzi Myoxocephalus* M±m = 17.0±0.6	*barenzi Gymnacanthus* M±m = 29.5±1.7	*cyclopteri Cyclopterus* M±m = 30.3±3.9
maris-albi Myoxocephalus (II) M±m = 13.5±0.7	t = 3.49 0.I%>P	—	t = 4.24 0.I%>P	t = 3.80 0.I%>P	t = 8.89 0.I%>P	t = 4.25 0.I%>P
maris-albi Myoxocephalus (I) M±m = 18.8±1.4	—	t = 3.49 0.I%>P	t = 6.07 0.I%>P	t = 1.22 5%<P	t = 4.84 0.I%>P	t = 2.80 0.I%<P<I%
maris-albi Myoxocephalus (III) M±m = 9.7±0.6	t = 6.07 0.I%>P	t = 4.24 0.I%>P	—	t = 9.36 0.I%>P	t = 5.45 0.I%>P	t = 5.03 0.I%>P
barenzi Myoxocephalus M±m = 17.0±0.6	t = 1.22 5%<P	t = 3.80 0.I%>P	t = 9.36 0.I%>P	—	t = 6.94 0.I%>P	t = 3.41 0.I%>P
barenzi Gymnacanthus M±m = 29.5±1.7	t = 4.86 0.I%>P	t = 8.89 0.I%>P	t = 5.45 0.I%>P	t = 6.94 0.I%>P	—	t = 0.199 5%<P
cyclopteri Cyclopterus M±m = 30.3±3.9	t = 2.80 0.I%<P<I%	t = 4.25 0.I%>P	t = 5.03 0.I%>P	t = 3.41 0.I%>P	t = 0.19 5%<P	—

большое количество ДНК в Ма. При увеличении содержания ДНК в Ми увеличивается и количество ДНК в Ма. Предположительно авторы объясняют это явление более или менее постоянным числом эндомитозов, которые претерпевают зачатки Ма в экскончюгантах при формировании окончательных Ма вегетативных особей. Возможно, что это предположение применимо и к триходинам, однако, оно нуждается в тщательной цитологической проверке на однородном (клональном) материале.

Резюме

Синтез ДНК в Ма *T. cottidarum* происходит в интерфазе. Количество ДНК удваивается в интерфазе, достигая максимума перед началом деления. Удвоение ДНК в Ма предшествует делению Ми. Ма недавно разделившихся инфузорий содержит минимальное для вегетативных особей количество ДНК. Ма ранних конъюгантов по содержанию ДНК мало отличаются от Ма вегетативных особей, что является одним из доказательств отсутствия у триходин преконъюгационного деления. Синтез ДНК в зачатках Ма, образовавшихся в результате деления синкариона, начинается очень рано. Еще до первого метагамного деления

экскончюгантов количество ДНК в зачатках в 15 раз превышает среднее количество ДНК в Ми вегетативных особей. Различие в количестве ДНК у разных зачатков одного экскончюганта предположительно объясняется неодинаковой скоростью синтеза ДНК в них. Обнаружены колебания содержания ДНК в вегетативных Ма как в пределах вида *T. cottidarum*, так и в пределах популяции. Содержание ДНК в Ма вегетативных особей *T. cottidarum*, не зависит от хозяина и географического распространения. Отмечаются различия в соотношении количества ДНК в Ма и Ми у разных форм и популяций *T. cottidarum* (242 : 1, 290 : 1. 376 : 1, 426 : 1, 758 : 1).

SUMMARY

Quantitative measurements of the DNA content in the macronucleus of *Trichodina cottidarum*, at different stages of the life cycle, were executed by photometric method in visible light at 546 mμ of wave length. The DNA quantity is doubled at the end of the interphase. The duplication of the DNA content in the macronucleus precedes the micronucleus division. The least quantity of DNA was found in the macronucleus just after division of the ciliate. The macronucleus of young conjugants is similar to that of vegetative animals, as concerns the DNA content. Duplication of DNA in macronuclear anlagen of the exconjugant is followed by the first metagamic division. The DNA content in macronuclear anlagen of young exconjugants was shown to exceed the mean DNA content in the vegetative micronucleus 15 times. The DNA content in the vegetative macronucleus of *Trichodina cottidarum* varies both on species and population levels. The quantity of DNA in macronucleus of vegetative animals of *Trichodina cottidarum* depend neither on their hosts nor in the geographical origin of the sample. The ratio of the DNA content in the macronucleus to that of micronucleus of the vegetative animals of *Trichodina cottidarum* were found to amount 242 : 1, 290 : 1, 376 : 1, 426 : 1 and 758 : 1.

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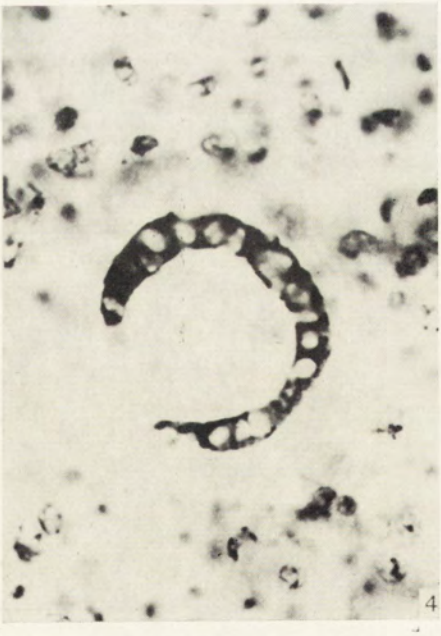
ПОДПИСИ К ТАБЛИЦАМ I — II

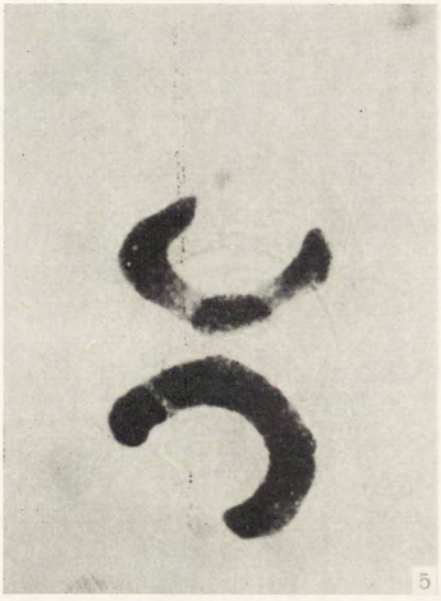
1 : Делящаяся клетка *T. cottidarum* с дочерними Ma. Окраска по Фельгену
2—4: Вегетативные ядра *T. cottidarum* на разных стадиях развития. Окраска по Фельгену
5: Начало конъюгации у *T. cottidarum*. Окраска по Фельгену
6: Ранний эксконъюгант *T. cottidarum*. Окраска по Фельгену
7—8: Разные стадии эксконъюгантов *T. cottidarum*. Окраска по Фельгену

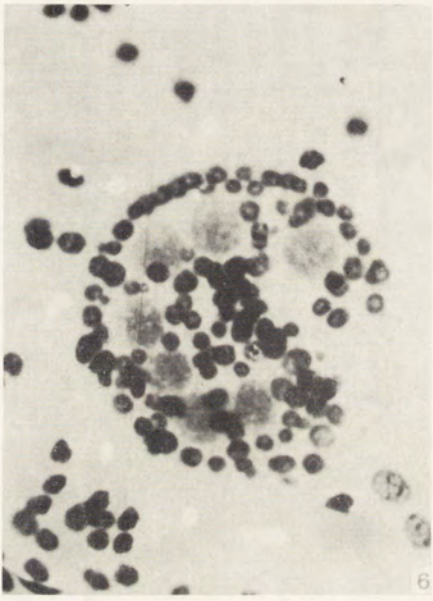
EXPLANATION OF PLATES I — II

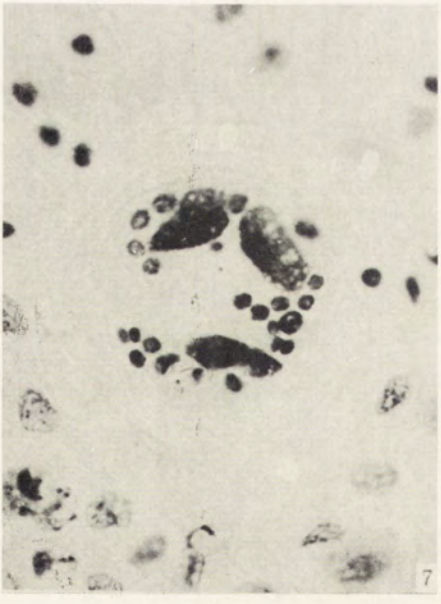
1: A dividing cell of *T. cottidarum* with daughter Ma. Feulgen stain
2—4: Vegetative nuclei of *T. cottdarum* on different stages of their development. Feulgen stain
5: Beginning of conjugation in *T. cottidarum*. Feulgen stain
6: Early ex-conjugant of *T. cottidarum*. Feulgen stain
7 — 8: Different stages of ex-conjugants in *T. cottidarum*. Feulgen stain

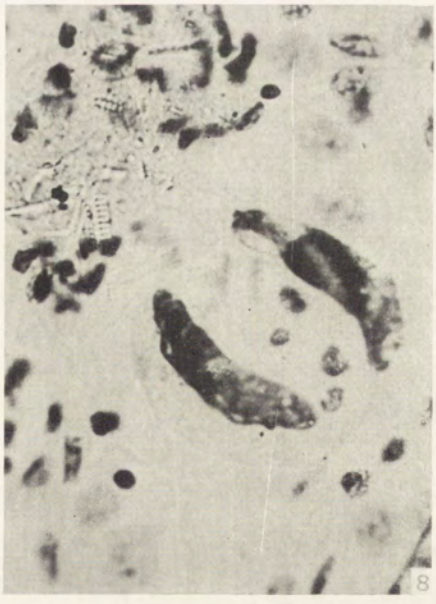


Г. А. Штейн auctor phot.









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Swimming and its ciliary mechanism in *Ophryoglena* sp.

Ophryoglena sp. mozgása és csillómechanizmusa

Protozoologists have shown increasing interest in Ophryoglenids after the publication of the comprehensive paper of Mugard 1949. In recent years several additional papers were published on their finer structure, mode of life, and systematics (Canella e Trincas 1961, Dragesco 1962, Grassé et Mugard 1961, Mugard et Renaud 1962, Savoie 1961, 1962 a, 1962 b), moreover several new species were described with due attention to modern taxonomic principles (Canella e Trincas, 1961, Savoie 1961, 1962 a, 1962 b). Attention was, however, focussed on the peculiar histophagous feeding of *Ophryoglena* (Mugard 1949, Savoie 1961, 1962 a, 1962 b, Dragesco 1962).

Much less is known concerning the normal locomotion of *Ophryoglena*, although their remarkably fast spiralling has been repeatedly reported. Bullington 1925 and Ludwig 1929 were among the first to present some observations on their movement. As the data required for the species recognition were not given in these papers and the systematics of *Ophryoglena* having undergone a thorough revision in the meantime, it cannot be ascertained at present whether the animals studied were real *Ophryoglena* or not.

As a part of a detailed study on ciliary activity in infusoria (Párducz 1954, 1956, 1959, 1961, 1964) certain data on normal movement and ciliature of an *Ophryoglena* sp. were obtained and will be reported below. The reactions of the same species to food will be described in a forthcoming publication (Párducz and Müller 1964).

Material and methods

Ophryoglena sp.[2] was obtained by Dr. M. Müller (Budapest) in 1961 from Dr. A. Savoie (Busigny, Nord, France). The animals were kept in Prescott solution (Prescott 1956) and fed on frozen rat kidney (Savoie 1961). In the present experiments theronts starved for one or two days were collected, and washed and studied in Prescott solution. Tracks of movement

[1] Deceased in February, 1964.

[2] Author expresses his sincere gratitude for obtaining this species.
The species is being described as *O. bacterocaryon* by Roque, de Puytorac et Savoie (Arch. Zool. exp. gen. in press). Personal communication of Dr A. Savoie to Dr. M. Müller.

were taken in a photomacrographic device (D r y l 1961). The ciliature was demonstrated by means of the osmic acid-haematoxylin method (P á r d u c z 1952).

Results

Animals which starved for 24 to 48 hours after emerging from the cysts formed by encysted tomonts represent typical theronts. The theront stage may persist in certain cases for 5 to 6 days without encystation. The oral groove (vestibulum and pharynx) are located halfway between anterior end and the equator of the animal. In contrast to *Paramecium* the theront has no deep and prominent peristome beginning at the left of the anterior end and extending obliquely across the body to the mouth. Thus this animal has an almost ideal rotation symmetrical shape. The body, 280 to 450 μ in length, is pear shaped with broadly rounded anterior end and tapering posteriorly and circular in cross section. In the first third it is somewhat expanded.

The entire body is covered by a coat of uniform single cilia arranged in longitudinal lines which converge before the mouth and form the short preoral suture turning slightly to the right. The postoral rows of cilia sharply turn to the rigth and thus reach the external part of the oral groove, i.e. the vestibulum. Cilia are somewhat shorter and thinner but are more densely arranged than in *Paramecium*. Ten adjacent ciliary rows in *Paramecium* correspond to 14 rows in *Ophryoglena* and 10 cilia of the same row in *Paramecium* correspond to 28 cilia in *Ophryoglena*.

It is well known that the characteristic normal movement of *Paramecium* is determined by the existence of the extensive oral groove which is deeply depressed in the prestomal area. As the activity of the prestomal cilia is not fully utilized for the purpose of locomotion, the steady dominance of the dorsal ciliature is characteristic for the *Paramecium*. Accordingly the freely swimming animals follow a regular spiral path instead of a direct line. In view of the regular symmetric form and the regular distribution of the almost meridional ciliary rows on the *Ophryoglena* theronts, more simple forms of normal locomotion could be expected in this case. If namely all cilia beat in the same direction and with the same force their action should result in a simple dislocation of the ciliate body along its longitudinal axis. Such almost straight paths of the theront can indeed be observed sometimes, but this locomotion is accompanied by a rotation of the body along its axis. This rotation is counterclockwise if seen from behind, i.e. to the left. Accordingly, the cilia do not beat, in this case either, to the rear end but obliquely and backward to the right. In three cases 2500, 3000 and 3600 μ/sec velocity was observed in such animals (Pl. I 1).

This straight locomotion cannot be observed too frequently. The theront follows in most cases a space curve, i.e. a left spiral, as observed earlier also by B u l l i n g t o n 1925, L u d w i g 1927 and C a n e l l a e T r i n c a s 1961. It makes a full turn during each period of spiralling and thus always the same side of the body is turned toward the axis of the spiral. Accordingly, a certain asymmetry characterizes most of the swimming of the animal. This asymmetry cannot be attributed to morphological features, as for instance to slight regional differences in the size or distribution of ciliature (e.g. the lack of locomotor forces in the area of the small oral grove or the convergence of ciliary

rows along the short preoral suture) because these would produce a single characteristic pattern of locomotion, whereas great variation in the slant and width of the spirals is observed even in the case of the same animal. The differences in individual paths may be attributed rather to certain physiological disproportion of the body surface, i.e. to the regional fluctuation of the magnitude of locomotor forces. Since the ventral surface is invariably turned toward the virtual axis of the spiral in every type of locomotion it is clear that their movement is dominated throughout by dorsal rowing forces.

Photographic tracking of locomotion provides more detailed information concerning the momentary distribution, magnitude and direction of locomotor forces.

According to the laws of geometry, to which spiral lines described by the *Ophryoglena* are also subject, a spiral line is the narrower and steeper, the faster its rotation along the longitudinal axis, the smaller the declination due to differences in the longitudinal force components and the smaller the asymmetry in the components of the rotatory force (Ludwig 1929). Plate I 2 and 3 clearly illustrate this law, the tracings show an increase of the rotation and a parallel decrease of the diameter and increase of steepness of the spiral.

Increased diameter is, however, not always accompanied by a decrease of steepness (Pl. I 5). In these cases the switch from one type of movement to another was most probably accomplished exclusively by an increase in the differences between logitudinal force components on antagonistic body surfaces. Cases, however, are frequent in which the distribution of both the rotatory and the longitudinal components is changed.

Ludwig 1929 showed that increased intensity of beating, i.e. faster locomotion, should not alter the diameter and steepness (pitch) of the spiral path if Stokes' law of resistance is valid for the movement of ciliates and if the beating plane remains unchanged. Schaeffer (cited after Bullington 1925), however found in *Paramecium* that lowering the temperature decreased forward movement and increased the number of spiral turns. This relation of speed to the number of spiral turns has been confirmed also by Bullington 1925 on different species, stating that greater speed is invariably associated with longer spirals and fewer spiral turns, while slow movement is associated with shorter spirals and more spiral turns. The latter observations thus suggest a regular shift of the beating plane of the cilia when movement becomes faster.

Present observation on *Ophryoglena* show, however, that the relation of the form of the spiral path to speed cannot be interpreted on the basis of a single scheme.

At one hand Plate I 4 shows identical steepness and diameter of the spiral in two animals travelling at different speeds. Probably this is the case, where, as assumed by Ludwig, there is an uniform increase in the beating intensity of all cilia without any change in the direction of beating.

On the other hand tracings on Pl. I 5 correspond to Schaeffer's and Bullington's observations. An increase of velocity can be seen in this set with parallel decrease of the number of spiral turns (of the speed of rotation) and with and increase of their pitch.

We must notice, however, that as in the case of the *Didinium* (Párducz 1961) the straight locomotion here is generally somewhat slower than the movement along a steep helical path. The greatest speed produced by theronts

was 4000 μ/sec. The paths followed were very steep, but still well recognizable spirals (Pl. I 2c and 4b). Thus these animals made in a second a distance amounting to 12 times their average length of 350 μ, while 6 or 5 complete turns were made during 2 secs.

No conclusions can be drawn, as to the rotation, from tracings of straight paths. But the markedly helical paths also show that the assumed definite relation between speed of the organism and the length of the spiral does not prevail. It is thus not a rule that exemplares moving with greatest speed swim in open spirals, with the fewest spiral turns, while those moving with least speed swim in close spirals with many spiral turns. The tracing on Plate I 5a shows also 6 turns during 2 secs although the animal made only 2500 μ/sec.

Yet further evidence of the independence of speed and shape of paths can be obtained by detailed study of the tracings. E.g. Plate I 7 shows paths made at the same velocity while there is a difference in the numbers of turns.

The comparison of the tracings on Plate I 8 is also interesting. While an identical distance (measured along the axis of the spiral) is covered in 2 secs. by each, there is a marked differences in the length and diameter of the turns. It is true that the diameter of the helix and thus the real length of the route covered is, in most cases, not the same, though axial speed be constant. A comparison of Plate I 9a and b shows, however, that an animal following a wider helix and making the same number of turns may cover the same distance a good deal faster.

Especial interest is attached to tracings which reveal the ability of the animal to change form of its movement even during locomotion. A most simple example is given when different distances are made during the same time intervals (as shown on tracings made with interrupted light), showing transient increase or decrease of speed (Pl. I 10a, b). Another frequent case is represented by temporary changes of pitch and diameter of the helix (Pl. I 10c, d).

The locomotion of *Ophryoglena* in pure Prescott solution, and especially if a small amount of food is added, is characterized by frequent distortions of the path, resp. frequent changes of direction or short back outs interrupting the uniform movement (Pl. I 11, and. II 15).

The most frequent and simple mode of changing the direction is a transition to a plane curve from the helical path. This motor effect is most probably performed by the rotation stopping along the longitudinal axis while dissymmetry in the longitudinal force components is retained (Pl. II 14a).

Further changes to be frequently observed are the topplings over of the fore end to all possible directions, most frequently to the ventral side, further short backward springs, not longer than a fraction of the body.

A most frequent phenomenon is a regular bend in the axis of the spiral path (Pl. II 12), which demonstrates a very sensitive adaptation of the ciliary activity (Ludwig 1931, Párducz 1954).

It is remarkable that reversion, i.e. backward swimming, so easily evoked in *Paramecium*, is very rare in *Ophryoglena* and is of very short duration. The animals' backward swimming never covers more than one or two body lengths.

It is doubtful whether arching without rotation and swimming along regular circular orbits occur (Pl. II 13 and 14). Plate II 13 shows most probably

a very wide spiral with a vertical axis after the animal arrived at and was impeded by the bottom. Plate II 14c represents most likely a hampered wide spiralling. The arches the animals performed are rather irregular alternatingly to the right and left in the plane of the image. It may be assumed that the animals, who were probably injured, glided just above the bottom and, when turning downward, repeatedly touched it and thus could not describe their regular spiral path.

If the animal meets a solid obstacle in course of its movement, the longitudinal force components cannot come into play and another type of forced movement appears. The animal rotates at the same spot along its longitudinal axis for a while. The sense of the rotation, just as that of normal movement, is counterclockwise.

In addition to the enumerated, rather simple changes of locomotion, the animal can produce highly different complex paths, too (Pl. II 14b). In general, the *Ophryoglena* shows a marked tendency to change frequently the direction of swimming. Probably a higher excitability of *Ophryoglena* explains why frequently changing, turning etc., paths are seen even in seemingly homogeneous medium (Pl. II 15), instead of the regular spirals produced by *Paramecium* under similar conditions. These complex paths can be explained only by assuming that the animal is capable of changing within wide limits the intensity and the direction of ciliary beating in any region of its body.

The very fast theronts do not reveal much detail of the ciliary activity. This explains the lack of corresponding data. Lower temperatures and, as will be shown in a subsequent publication (Párducz and Müller 1965), presence of food, lead to decreased velocities thus permitting the observation of the rapid forward extension of metachronal ciliary waves from the posterior end of the animal.

Fixed and stained preparations display much more details. Observation under low power reveal alternating thicker light and thinner dark stripes (Plate III 16), winding in a left spiral around the body. Under higher power the stripes can be seen to be due to rhythmic alternations of different fixed stages of the beating cycle (Plate III 18). The dark areas are formed by the crowding of cilia in the end phase of the effective stroke or at the beginning of the recovery, whereas the light areas contain the straight cilia fixed during the performance of the effective stroke. The fixed positions of the cilia allow a detailed analysis of several characteristics of the ciliary activity. They show that the plane of the effective stroke is perpendicular to the surface and is approximately parallel with the ciliary crests, i.e. has a posterior right direction. This observation is in agreement with the left spiralling of living animals. The subsequent phases within a beating cycle follow in succession toward the posterior end which is in agreement with the forward propagation of metachronic waves in living animals. During the recovery phase the cilia lie almost parallel to the body surface and rotate toward the starting position counterclockwise. During this movement they have an arched form with the convex side turned to the right of the animal.

Animals swimming along straight paths or describing very extended, narrow spirals show parallel, regular waves which are evenly spaced and form a 45° angle with the body axis (Pl. III 16—18). This pattern extends also over the mouth region where no specialized arrangement is observed.

In general the ciliary pattern is highly variable on fixed animals. The number, spacing and angle of waves vary even from region to region (Pl. II 19—21). This is again in good agreement with the observed variability of locomotion.

Discussion

Living animals and especially the fixed preparations clearly show that the metachronic waves start at the posterior end and extend forwards along the body. It is further demonstrated that the plane of the effective stroke does not coincide with that of the regressive phase. The effective stroke is parallel with the crests of the waves and to the right in respect of the direction of the wave propagation. These facts allow the assumption that the metachronally coordinated ciliary activity, as in other species, is the combined result of two, essentially spontaneous rhythmic processes: 1. the uniform counterclockwise rotation of the individual cilia, and 2. a series of rhythmically propagating impulses of excitation which transform the apolar rotation of the individual cilia into effective strokes which appear with a definite phase shift, have a certain direction and consist of progressive and regressive phases. Accordingly, the same type of coordinated ciliary activity was found, which was first described in *Paramecium* and *Colpidium* (Párducz 1954, 1965) and found in several other ciliates to date. The only difference is that a greater number of the more closely arranged cilia are involved in the progressive and regressive phases and somewhat greater number of waves, in general 16 to 20 metachronal waves, are seen simultaneously on the body surface, since the animal is larger.

The cilia of *Ophryoglena* are closer together than those of *Paramecium*. At the same time many more (about twice as many) cilia are involved in the formation of each metachronal wave. Notwithstanding this fact the conduction process is not slower in the *Ophryoglena* than in *Paramecium* and the wave length is of the same order in both cases. This points to an independence of the wave velocity on the number of cilia. This conclusion does not favour a conduction mechanism in which the cilia themselves take part (this would hold true even if the *Ophryoglena* cilia were beating with higher frequency).

A still widely accepted view concerning the locomotion of ciliates holds that their action system (possible changes in the ciliary activity) consists of a very limited number of different activities, all of which are based on uniform action of the total ciliature. Both the tracings and the fixed preparations show, however, that *Ophryoglena* possess a wide range of possibilities in motor regulation. Its movements show sensitive adaptations to the changing situations of the milieu since both the direction and the power of the effective stroke can be changed in any individual body region and at any time. This adaptation is especially clearly demonstrated in feeding animals (Párducz and Müller 1965).

According to Jennings' theory, the orientation of the protozoa in a stimulus field is the result of repeated performance of a negative avoiding reaction, i.e. it is result of trial and error. The animals have no positive reactions at all. Seemingly positive reactions are indirectly produced by accumulation of the single possible negative reactions. The animals simply cannot leave the optimal or indifferent area of the milieu because all movements which would

lead the animal out of this area result in unfavourable changes of the strength of the stimulus and necessarily in an avoiding reaction. In *Ophryoglena*, however, avoiding reactions were seen but very infrequently. Instead of this rigid and undirected reaction a great variety of simple, direct and thus rational responses were observed. In vivo studies revealed no "trials" at the margin of the stimulus zone. The animals returned to the optimal or indifferent zone usually by a single change of locomotion based on local changes of their ciliary activity.

Summary

The ciliary mechanism in the theront of the *Ophryoglena* species studied corresponds to the *Paramecium*-type: the metachronal waves propagate in a posterio-anterior direction, the effective stroke to right and backwards is parallel to the ciliary crests, the cilium bends out of the plane of beating and rotates counterclockwise almost parallel with the body surface until ready for the next stroke. This ciliary activity propels the freely swimming animals along a left spiral. Maximal speed of the animals is over 4 mm/sec. External stimuli may induce different local or total modifications of the ciliary activity which enable the theront to perform various forms of locomotion. The avoiding reaction plays a minor role in the action system of the animal. Orientation in a stimulus field is performed mainly by partial and direct modifications of the ciliary work.

ÖSSZEFOGLALÁS

A vizsgált *Ophryoglena*-faj therontjának csillómechanizmusa megfelel a *Paramecium* tipusnak: a hátsó testvégtől előrefelé tartó metachronikus hullámok, a hullámok frontvonalával párhuzamos, jobbra hátrafelé irányuló aktiv kicsapás. A regressziv fázisban a csilló kitér a csapás sikjából s a testfelülettel nagyjából párhuzamosan balra körözve kanyarodik vissza a készenléti helyzetbe. E csillótevékenység hatására az állat akadályozatlan helyváltoztatás esetén balracsavarodó spirális mentén uszik a viztérben. Maximális mozgássebesség 4 mm/sec. fölött. Külső ingerek hatására a csillótevékenység tág határok között és lokálisan is módosulhat, s ennek eredményeként a theront rendkivül változatos mozgásformák kifejtésére képes. Az állat akciósrendszerében a kitérési reakció alárendelt jelentőségü, az ingermezőben való tájékozódás elsősorban a csillók müködésének részleges és közvetlen befolyásolásán alapszik.

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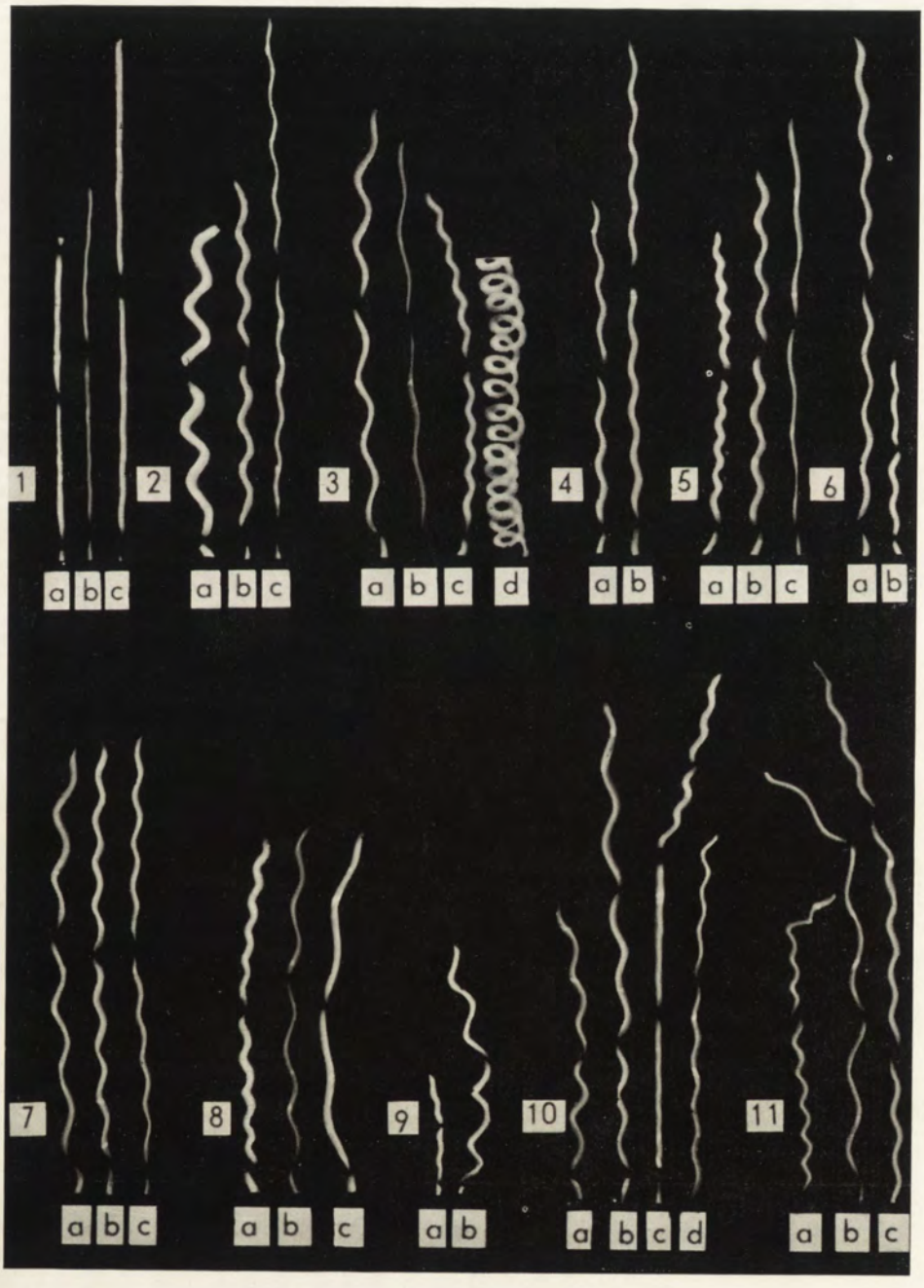
EXPLANATION OF PLATES I—III

1—15: Tracings of swimming *Ophryoglena* theronts. See text for further explanation. Break of light, if done, in every two secs (×5).

16—21: Metachronal ciliary pattern and its variability on theronts. Osmic acid—haematoxylin method (×320, except 18: × 650).



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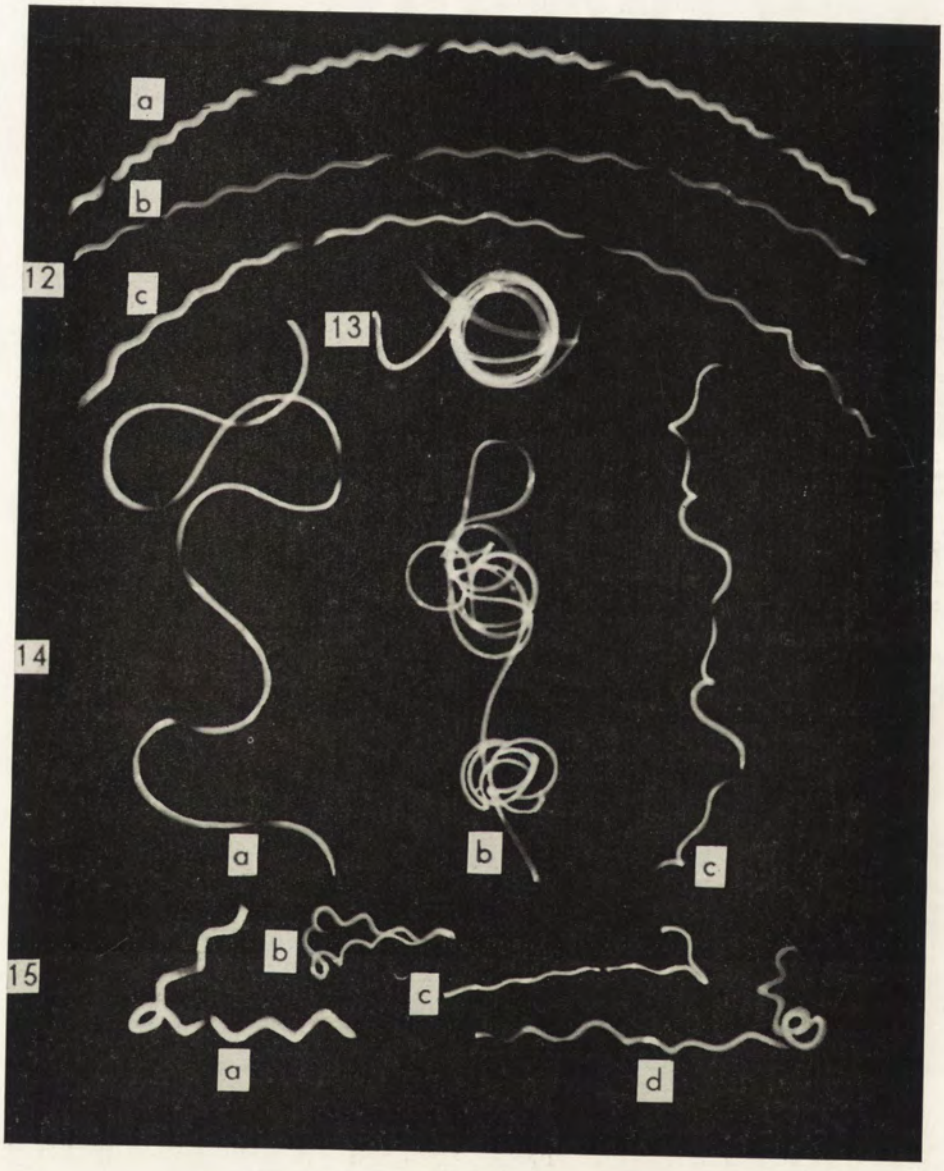

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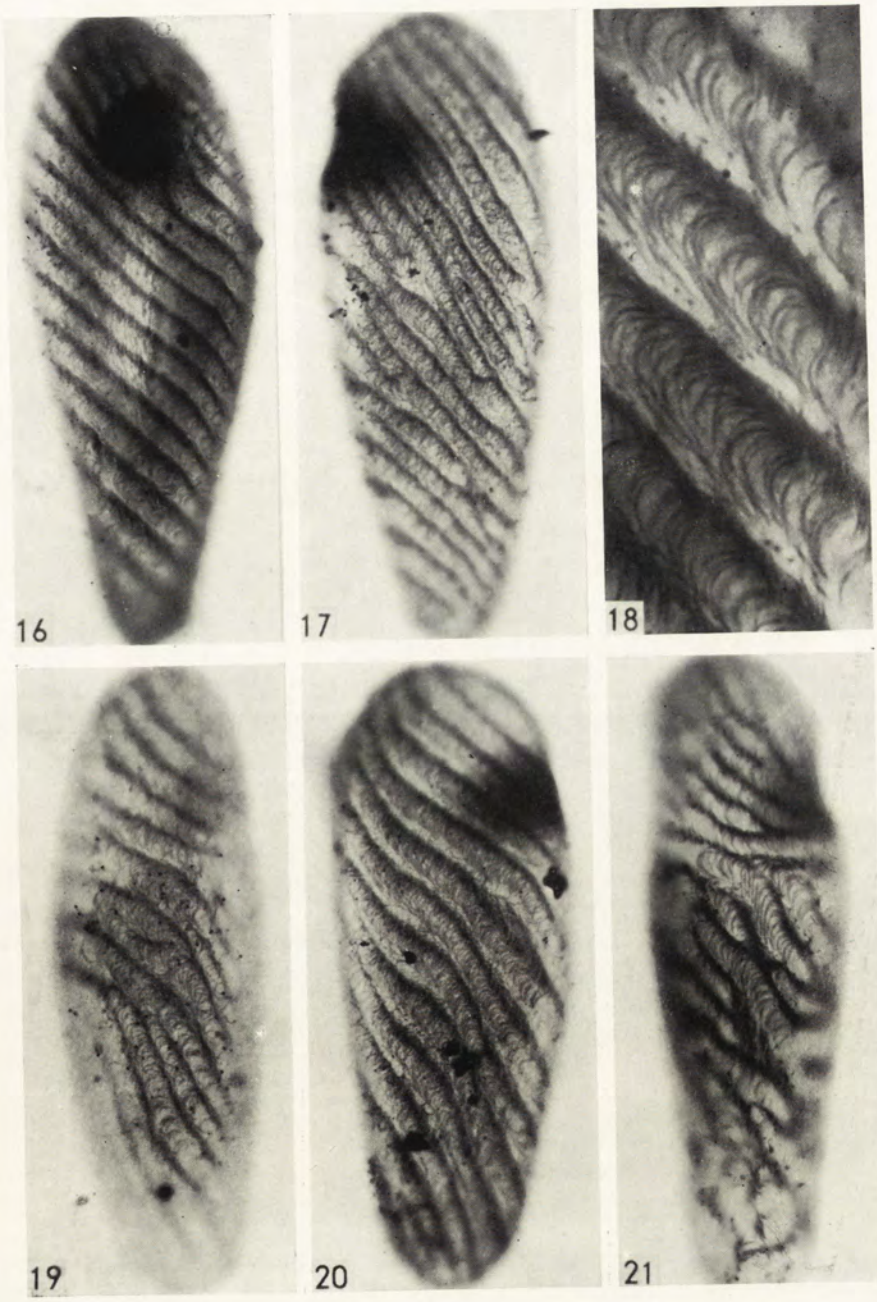

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Calcium substitution in staining the cilia

Podstawianie wapnia przy barwieniu rzęsek

The dimensions of cilia and frequency of their beating make a direct observation of ciliary function extremely difficult. It is therefore convenient to arrest these organelles in course of their work for the subsequent analysis of fixed stages of the ciliary stroke and of the mutual relationships of stages exhibited simultaneously by neighbouring cilia. In this respect the rapid fixation of protozoan cells with osmium tetroxide, introduced by Párducz 1952, gives excellent results preserving the true functional shape of each cilium and the pattern of metachronal waves; this technique allowed many essential findings in the further Párducz's research. The same fixation mode was used, subsequently, also for ciliated tissues and for an electron microscope analysis of position taken by working cilia (Satir 1963).

Nevertheless, the light microscope examination of whole mounts is highly uncertain because of the inconvenience of staining technique recommended by Párducz 1952. It consists in treating the fixed ciliates with some iron alum (5%) and staining them with haematoxylin (0.5%). It is very difficult to obtain well contrasted mounts in this way. In most cases, when cilia become sufficiently black, the cell body also darkens in a degree impeding the examination. Very numerous preparations are entirely failing, and even in a good mount only some percentage of cells are fit to be analysed. Obviously, no interpretation may be conclusive, when it remains unknown, whether a picture observed in rather few suitably stained individuals, really represents the pattern typical of the ciliary behaviour under study. Therefore, it becomes necessary to perform a very large number of preparations.

It may be presumed that the imperfection of classical haematoxylin staining of cilia is due to the fact that it seems to be connected with the formation of calcium lake. Calcium ions are indeed present in cilia, however, their concentration is also very high in the protozoan cell body (Akita 1941, Yamaguchi 1963), being the greatest just in its cortical layer (Lansing 1938).

Experiments proved this supposition to be correct. The body and cilia of *Paramecium caudatum* fail to stain with haematoxylin in presence of EDTA or other calcium-chelating agents. When EDTA is applied only after the osmium fixation, and Paramecia are subsequently rinsed before staining with a calcium-rich medium, haematoxylin stains the distal ends of cilia; the cell body in which calcium is chelated, as well as the proximal parts of cilia influenced by the diffusion of EDTA, remain colourless. The diffusion

of EDTA outwards of the cells may be stopped if the alum bath is interposed between the EDTA treatment and the passage into the Ca-containing medium. Then, the picture becomes satisfactory, i. e., transparent Paramecia with stained cilia are obtained.

Moreover, the results are much better, when calcium is substituted for another, more selectively adsorbing cation. Just recently it was revealed in several studies (Seravin 1962, Párducz 1962, Puytorac, Andrivon et Serre 1963, Kuźnicki 1963) that a very specific influence is exerted on cilia by nickel ions. On the other hand, it is well known (cf. Pearse 1953) that nickel also forms a black lake with haematoxylin. Finally, it was stated that the most suitable procedure for staining the osmium-fixed cilia consists in decalcification of cells with EDTA, their impregnation in alum solution and, after washing, staining with haematoxylin in the presence of nickel ions.

The details of the procedure, as aplied by the author, are the following:

1. Rapid fixation (after Párducz 1952) in solution of 2 g. OsO_4 + 0.5 g. $HgCl_2$ in 100 ml. H_2O; 5 min. (washing).
2. Decalcification in 0.02 M (ca. 0.75%) EDTA; 10 min. (no washing).
3. Impregnation in 0.5 M (ca. 25%) $Fe(NH_4)(SO_4)_2 \cdot 12H_2O$; 5 min (rapid washing).
4. Incubation in 0.02 M (ca. 0.5%) $NiCl_2 \cdot 6H_2O$; 1 min. (no washing).
5. Staining with haematoxylin (2 g. of haematoxylin + 0.5 g. $NaIO_3$+10 g. of ethyl alcohol in 100 ml. H_2O) added to the sample in 1:1 volume ratio; 5—15 min. — microscopic control is quite possible and recommended (washing).
6. Dehydration (after Párducz 1952) in alcohols: 30%, 50%, 70%, 96%, 100%, 100%, in carbol-xylene, xylene; embedding in balsam.

The described procedure gives excellent results, always allowing the examination and demonstrating as well the general pattern of ciliary cloth on the whole body surface (Pl. I 1), as the conformation of metachronal waves (Pl. I 2—3), and even the highest magnification of individual cilia kept on different stages of their work (Pl. I 4). As follows from photomicrographs published by Párducz and from the author's own preparations, the best picture achieved with the classical technique of Párducz 1952 may be not inferior to those obtained with calcium substitution. However, a perfectly stained individual in the classical procedure must be found out among many hundreds specimens. After decalcification and staining in the presence of Ni-ions — as to the author's sufficiently long experience — no preparations are failing, and in each sample the number of individuals fit for a detailed examination and taking pictures attains the level of 90%. It gives a certainty that the fixed stages of ciliary stroke, even if observed in a single mass preparation, are related to the investigated physiological state of the moving cell, and are not an incidental feature of few fortuitously selected individuals.

Summary

The author describes an improvement of the technique of staining the cilia after their rapid fixation with osmium tetroxide. It is based upon a decalification of the fixed material and, thereafter, its staining with haematoxylin in the presence of nickel ions.

STRESZCZENIE

Autor opisuje ulepszoną metodę barwienia rzęsek po ich szybkim utrwalaniu czterotlenkiem osmu. Polega ona na odwapnianiu utrwalonego materiału, a następnie barwieniu go hematoksyliną w obecności jonów niklu.

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EXPLANATION OF THE PLATE I

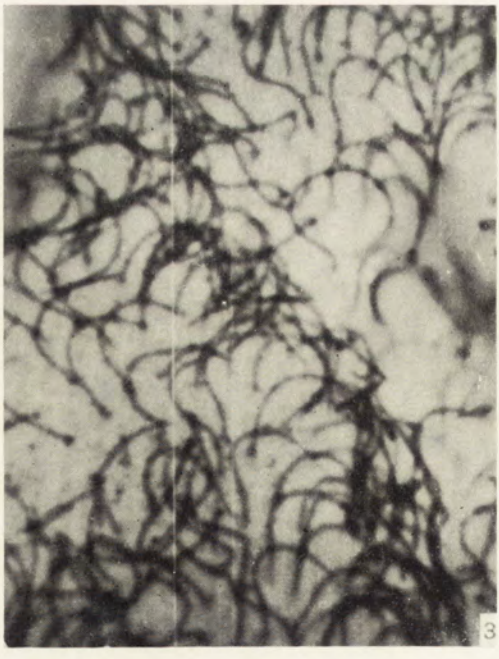
Cilia of *Paramecium caudatum* in normal movement, after osmium fixation and staining following the calcium substitution technique

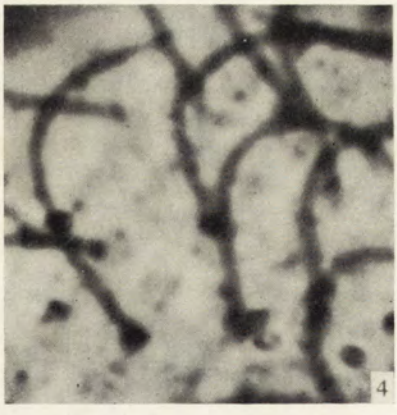
1: Fragment of a mass preparation demonstrating differences in the focus only, not in staining of ciliature (× 500)

2: Metachronal waves on the anterior part of an individual (× 1000)

3: A single ciliary wave (× 2000)

4: Individual cilia (× 5000).





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Modern lines in the study of amoeboid movement

Współczesne kierunki badań ruchu amebowego

Interpretation of the mechanism of amoeboid movement is a task transgressing far beyond the scope of physiology of amoebae. Amoeba is representing in this case a rather convenient natural model which is to be helpful in solution of the great cytophysiological problem — the formation of the cytoplasmic streaming inside the cell. This concerns both fundamental types of the cytoplasmic streaming: the amoeboid movement and cyclosis. Of course, the amoeboid movement theories mostly contribute to the elucidation of the locomotion in other *Sarcodina*, slime molds plasmodia, some creeping plant and animal gametes, blood cells, macrophages, fibroblasts, Schwann cells and many other cells in tissue culture. All that are cell movements since the linear direction of the endoplasm streaming, towards one of the poles, involves shifting of the whole cell. Nevertheless the recent achievements prove a possibility of a still more extensive synthesis: the fundamental principles of the amoeboid movement are closely connected with the elementary background of the typical intracellular movements — mainly the rotational cytoplasmic streaming (cyclosis) — although in this second case the whole cell remains motionless and no influence of its polarity upon the cytoplasmic streaming is observable.

In 130 years of research on the amoeboid movement the most important discoveries were always connected with the concepts explaining the formation of streaming basing on the cytoplasm contractility. This was the view of the pioneers of protozoology (Ehrenberg 1830, 1832, 1838 and Dujardin 1835, 1838), within this concept Schulze 1875 gave his first correct description of the amoeboid movement and dynamic organization of amoeba. On this assumption the most perfect of the classical theories: the concept of the tail contraction (Pantin 1923, and Mast 1926), was based. This concept was in progress every time when new data on structure of cytoplasm seemed to bring some concrete meaning to the general idea of contractility and to indicate its morphological ground. The early contractility theories were connected with the theories of the reticular structure of cytoplasm, postponed together with them in the period of domination of the alveolar theories, revived with the development of the concept of the colloidal properties of cytoplasm. Similarly, the present exuberant development of the contractility theories of the amoeboid movement is connected with the pro-

minent discoveries in biochemistry and submorphology of the fibrillar proteins as well as of the endoplasmic reticulum.

On the other hand, it is historical fact that the contractility theories of the amoeboid movement explain the locomotion itself better than they explain the causes of the changes of its character, i.e. the character of reacting to stimuli. It involved considerable interpretation difficulties in the past, and then in the transitory period of domination of the surface tension theories — led to the complete rejection of the contractility theories. This remains their disadvantage till present time. The considerable number of competing theories of the amoeboid movement is involved mostly by the fact that a synthesis, embracing not only the contraction as response of the cell inside, but also the excitation processes initiating on the cell surface, has never been put forward.

The history of the theories of amoeboid movement show significant tendencies which are of interest till present time, but it is not the author's intention to discuss the historical aspect of the problem. An excellent revue of earlier work has been published by De Bruyn 1947, but an article summarizing what was achieved and suggested since 1950, is missing. The articles of Allen 1961a and Wohlfarth-Bottermann 1963b, and 1964a, b concern rather some selected problems. Nevertheless, even the first years of the second half of the XX century brought more essential discoveries than its whole first half, and they gave ground to a number of competitive theories. It seems desirable to review them and to analyse whether they really are essentially contradictory or — on the contrary — they are concepts complementing in some way one another while leading to the future synthesis.

The author is greatly indebt to Professor K. E. Wohlfarth-Bottermann (Bonn) for offering the original electron micrographs of amoebae and slime mold plasmodia, for this article. Sincere thanks are also due to Prof. R. D. Allen (Princeton), R. J. Goldacre (London), N. Kamiya (Osaka) and J. L. Kavanau (Los Angeles) for their kind permissions to redrawn some of their figures.

Current data and ideas

Background of the contraction

In the past years, the most adequate amoeboid movement theories could not represent the concrete idea of contraction but bound the contraction activity of amoeba directly with the sol-gel cycle in its cytoplasm. Hyman 1917 claimed that the property of protoplasm called contractility is just the gelation of the colloidal solution. According to Pantin 1923, in the posterior closed part of the gelated tube — as the ectoplasm is — syneresis of the gel occurs, involving passage of water into endoplasm while ectoplasm is contracting and exerting pressure upon the content of the tube. Simultaneously, in the anterior part of amoeba, swelling occurs, i.e. imbibing water released by syneresis from behind. In the posterior part, syneresis is connected with solation of gel, while the anterior swelling — with gelation of sol (cf. Fig. 1). The most accepted theory of Mast 1926, 1932 is really only a modification of the Pantin's concept. Mast sees the essence of the motive force only in the contraction of the elastic gel; consequently he lo-

calizes the force exclusively in the rear of amoeba. From that region sol would be constantly pressed forwards.

The notion of the mechanism itself and the physicochemical basis of contraction remained in a high degree obscure. Only in the last years it became clear that the cytoplasmic contraction resulting in the amoeboid movement (as well as in other movements inside the cells) is the function of fibrils of an actomyosin-like protein, occurring at the expense of the ATP energy.

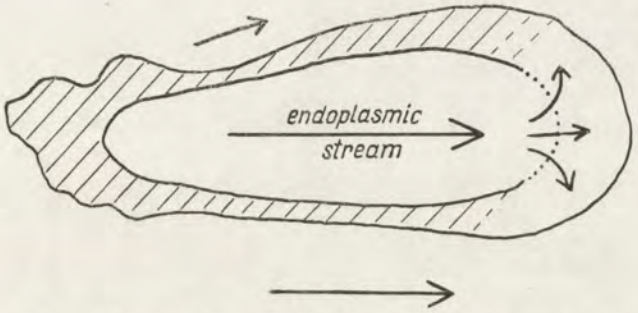

Fig. 1. Generalized scheme of the mechanism of amoeboid locomotion, after Pantin 1923

This problem was first and most exactly investigated in the slime mold plasmodia. The actomyosin-like protein was discovered by Loewy 1952 in *Physarum polycephalum*. Ts'o et al. 1956 a and b, 1957 a and b and Nakajima 1960 determined its properties more precisely as nearest the characters of the smooth muscle myosin B. This protein is usually called myxómyosin. Kamiya, Nakajima and S. Abe 1957 as well as Takata 1958 stated that ATP — administered externally or by injection — increases the motive force of the cytoplasmic streaming in plasmodium. The specific SH-inhibitors, as e.g. monoiodacetate and dinitrophenol (Kamiya, Nakajima and S. Abe 1957), act inversely. The parachlormercuribenzoate (PCMB) not only inhibits the motion but also lowers the enzymatic activity of the plasmodial actomyosin (Nakajima 1960). The ATP content in plasmodium was determined by Hatano and Takeuchi 1960.

Similar data were obtained lately for cells with rotational cytoplasmic streaming. Hatano and Nakajima 1959 (after Kamiya 1960) determined chromatographically the ATP content in the cells of *Nitella*. The influence of ATP, stimulating the rotational motion of cytoplasm, was also stated in *Nitella* (Sandan 1959) and in *Acetabularia* (Takata 1958). Inhibition of the rotational streaming after blocking the SH groups by PCMB and some other halogenated derivatives of the benzoic acid and its resumption after administration of cysteine was stated by many authors in *Nitella*, *Elodea*, *Hydrocharis* and *Tradescantia* (Smirnova 1955, S. Abe 1959, Kishimoto and Akabori 1959, Brueske and Applegate 1962). Recently the first report appeared on the extraction of an actomyosin-like protein from *Nitella* cells (Vorobeva i Poglazov 1963); it has been called algomyosin.

There is presently no doubt that the reaction of the actomyosin-like protein with ATP is also responsible for the contraction activity of amoebae themselves. Goldacre and Lorch 1950 stated the stimulating action of ATP injections upon the endoplasm streaming in pseudopodia. Also ATP introduced into the medium raises the viscosity of cytoplasm in amoeba (Kriszat 1952) and accelerates its movement (Käppner 1961); as known, it is first ingested by amoeba and hydrolized (Zimmerman 1962). The typical consequences of blocking and restoring SH-groups are also known for amoeba (Kriszat 1954, Zimmerman, Landau and Marsland 1958, Zimmerman 1959, Käppner 1961 b, S. Abe 1963). Investigations concerning the sol-gel cycle in amoeba cytoplasm became in the present also connected with the responses of fibrillar proteins to ATP (Landau, Zimmerman and Marsland 1954).

A special way of demonstrating the role of actomyosin and ATP in the amoeboid movement is performing of the so called fibrillar models by extraction the cells with glycerol or saponin and preserving only the framework of the fibrillar proteins. Such models contract in presence of ATP. This method, worked out by Szent-Györgyi 1949 for muscle fibres, was subsequently applied successfully for the mitotic spindle and for furrows dividing the cell body (Hoffmann-Berling und Weber 1953) as well as for cilia of *Ciliata* (Hoffmann-Berling 1954, Seravin 1961). The first fibrillar model of the cell showing an amoeboid movement was a model of fibroblast and of an undefined species of amoeba, reacting to ATP (Hoffmann-Berling 1958). In the case of amoebae, this procedure could not be repeated successfully at first, despite attempts made by several authors. Finally Simard-Duquesne and Couillard 1962 a and b succeeded in obtaining as well the glycerol-extracted model as the biochemical preparation of myosin from *Amoeba proteus*.

The third group of findings proving the activness of contractile proteins to be the motive force of amoeboid movement, originates from the submicroscopic morphology. Unfortunately this documentation is most scarce. No fibrillar structures could be detected in cytoplasm of slime molds plasmodia by Stewart and Stewart 1959 b using the electron microscope. Recently Wohlfarth-Bottermann 1962 found fibrils in the ectoplasmic region of *Physarum polycephalum* (Pl. II 3); they fail to be detected in the fresh sampled drops of plasmodia endoplasm, but they are formed there 10 min. after sampling, and then their dimensions correspond to the size of myxomyosin threads extracted from *Physarum* by T'so et al. 1957 b. Lately conditions were also found permitting the observation of slime molds fibres by means of the light microscope (Wohlfarth-Bottermann 1963 a).

Also the submorphological substrate of the rotational movements of cytoplasm is emerging. Jarosch 1958 found moving fibre bundles, seen in the light microscope, in the internodial cells of *Chara*.

Amoebae and some other *Sarcodina* present still more difficulties. Fibrils disposed in bundles were found at first by means of electron microscope at the base of axopodia in the heliozoan *Actinosphaerium*, by Anderson and Beams 1960. Other bundles were much earlier described in the light microscope in *Acantharia*.

Goldacre 1961 observed in phase contrast, and produced by means of a micromanipulator, fibres from the *Amoeba proteus* ectoplasm, after its partial dehydration. He considers them to be bundles of the elementar contractile fibrils. Until quite lately, occurrence of fibrils in amoebae sensu stricte, as revealed by the electron microscope, was reported only by Lehmann 1958. They were detected closely beneath the plasmalemma and also around the contractile vacuole. This finding was looked upon as artifact by majority of authors (cf. Pitelka 1963). Also the difference between the ectoplasmic gel and endoplasmic sol in amoebae could not be noticed in electron micrographs for a long time. The cause of failure was an exceptional fragility of cytoplasmic structures in amoebae and also the fact that the three-dimensional network can scarcely exhibit its fibrillar character, because the longitudinal sections occur rarely while the oblique and cross sections dominate.

Those difficulties have been overcome in the recent studies of Wohlfarth-Bottermann 1960, 1963, and 1964, on amoeba *Hyalodiscus simplex*. The endoplasm proved to be rich in membranous and vesicular elements of the endoplasmic reticulum, embedded in a homogenous matrix, with no fibrillar structures. The ectoplasm consists of pure matrix containing filamentous elements (Pl. I 1, II 2).

Generally it may be stated with full conviction that the intense study of the last ten years showed that the activity of cytoplasm being the motive force of the amoeboid movement is nothing else but the contraction of a fibrillar protein of actomyosin type, occurring at the expense of ATP energy, which is characteristic for muscles. More so, it became clear that the fundamental unity of the motory mechanisms at the molecular level concerns not only the one-direction cytoplasm streaming in slime molds and amoebae, as well as in other cells able to amoeboid movement, but also the ciliary and flagellar movements, rotational movement in plant cells, and even — very likely — cytokinesis and karyokinesis in mitosis.

Ectoplasm contraction in the tail region

Detection of the role of the actomyosin-ATP system in the cytoplasm of amoebae and slime molds plasmodia caused a great progress and a natural domination of the contractility theory of the amoeboid movement. Several variants of this concept are possible. Following the former views on the bipolarity of amoebae (Dujardin 1838, De Bary 1864, Pantin 1923) and accepting the classical thesis of contractility of plasmagel, it would be possible either to put forward the concept of the motoric role of contraction of ectoplasm in the posterior part of amoeba, or of its extension from forward. Recently also the hypotheses of contractility of plasmasol in amoebae have been proposed (Seifriz 1953 and Allen 1961 a and b). So the concepts looking for the endoplasm contraction anteriorly or its posterior extension also would be conceivable. Out of those four eventualities: posterior ectoplasm contraction, posterior endoplasm extension, anterior endoplasm contraction, and anterior ectoplasm extension — the extension concepts are considered by nobody. They seem so little probable on account of all the data on biochemistry of muscle proteins, indicating that the effective work in each case is performed by the contraction of the fibrillar protein but never by its relaxation. There then remains only the theory of the plasmagel con-

traction in the rear of amoeba and of contraction of plasmasol at the tip of the advancing pseudopodium.

The first concept is the most natural continuation of both early theories of contraction, that were motivated best, presuming that the rather liquid endosark is pushed forwards by the pressure of the posterior part of the ectosark (Schulze 1875 and Mast 1926). The actual view is only rather supplementing the former morphophysiological description explaining its mechanism on the molecular level.

So e.g. the former sol-gel cycle theory has been transformed in this line owing to the studies of Marsland and his co-workers. Landau, Zimmerman and Marsland 1954 conceive the ectoplasmic gel as a three-dimensional network of contractile protein fibrils. As it is proved by the distinct gradient of solation under the action of high pressures, the gel contractility is higher in its posterior "older" parts than in the anterior ones, newly formed from the sol. For that reason ATP evokes the ectoplasm contraction forcing forwards the endoplasm — just in the posterior part of amoeba[1]. The reverse process — gelation of endoplasm at the head of pseudopodium — would occur under the influence of ATP as well. This last idea seems to be especially interesting compared with the discovery of Marsh 1951, that ATP in the muscle is also a plasticizing relaxation agent, if not hydrolized.

The largest modern theory of the amoeboid movement, based on the contraction of the posterior parts of ectoplasm, was developed by Goldacre (Goldacre and Lorch 1950 and Goldacre 1961). According to Goldacre the protein molecules in the endoplasmic sol are folded, whereas in the

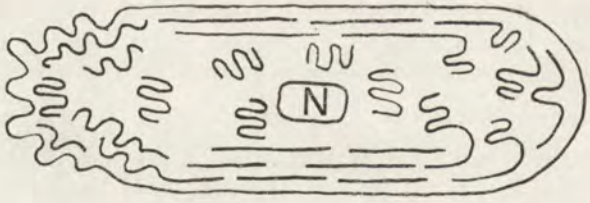

Fig. 2. Scheme of the molecular mechanism of ameoboid movement, following Goldacre and Lorch 1950. Unfolded protein molecules are present in the peripheral (ectoplasmic) layer, the folded ones — in the axial (endoplasmic) zone. Folding (contraction and solation) is indicated in the tail region, and the unfolding (gelation) — in the frontal region. N — the nucleus

ectoplasmic gel they are unfolded and form a network of fibres. Gelation of sol at the tip of the extending pseudopodium consists in unfolding the protein molecules. Solation of gel in the tail of amoeba would begin by the contraction of the fibrillar protein and terminate in further folding of its mo-

[1] Just recently the gradient of surface elasticity was also found in *A. proteus* by Kanno 1964 b.

lecules till the globular form, specific for sol, is formed (Fig. 2). Goldacre considers the contraction and solation of the ectoplasmic gel to be a reaction of the actomyosin-like protein with ATP.

Consequently, the posterior contraction of amoeba is as well the source of energy of the endoplasmic streaming as the source of material which it carries. Moreover, during the gel contraction, the water solution leaks out of it as of a sponge and — after pushing forwards — form a hyaline cap at the head of the advancing pseudopodium. At last, the posterior contraction of amoeba performs an osmotic work: the unfolded protein molecules of the gel, owing to the free side chains, have more adsorption sites than the folded sol molecules; as result, the various substances from the medium may be adsorbed by the ectoplasmic gel, and then they must be desorbed in the cell interior, in the course of solation.

Those concepts — reviewed above are supported by experiments concerning the way of dyestuff absorption by amoebae (Goldacre and Lorch 1950, Goldacre 1952, and Prescott 1953), influence of ATP injection, or injection of an anti-coagulation factor — heparin (Goldacre and

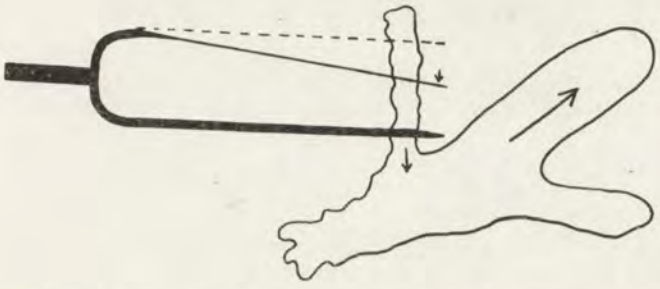

Fig. 3. Bending of a microbalance inserted into a retracting pseudopod. A technique which allows not only to demonstrate but even measure the force of contraction (after Goldacre 1961)

Lorch 1950). He collected also evidences suggesting that the posterior part of amoeba is contracting really. An interesting microchirurgical method of illustrating this fact is represented in Fig. 3.

Goldacre 1961 denies the existence of the rolling movement of plasmalemma, which was suggested by Jennings 1904 and accepted by Mast 1926. By a number of ingenious experiments he tries to base the view that plasmalemma is resting, being continuously resorbed in the contracting posterior part of amoeba, and with no interruption re-built in the frontal part. Very skillful in its simplicity is the Goldacre's experiment showing the immobility of the glass wool fibres covering the moving amoeba (Fig. 4).

A supplement to the theory of Goldacre is his concept of the feedback control of the velocity of the amoeboid movement (Goldacre 1956, 1957, and 1961). Goldacre presumes that the contraction of proteins at the expense of ATP energy is released enzymatically by the cell membrane, consequently it may occur in the area of contact of ectoplasm with plasmalemma. He also accepts the real existence of the hyaline layer separating plasmalemma from ectoplasm, invisible in the electron microscope. In con-

trast to Mast 1926, he presumes that this layer extends not so far as the posterior end of amoeba. Just for that reason the contraction occurs just in the tail and in no other place. The broader the contact area of the membrane and plasmagel is, the stronger is the contraction evoked. Then it involves a more significant production of hyaline, the hyaline layer extends, the contact area becomes limited and, finally, this feed-back mechanism involves the fall of the contraction activity after every contraction stronger than usual.

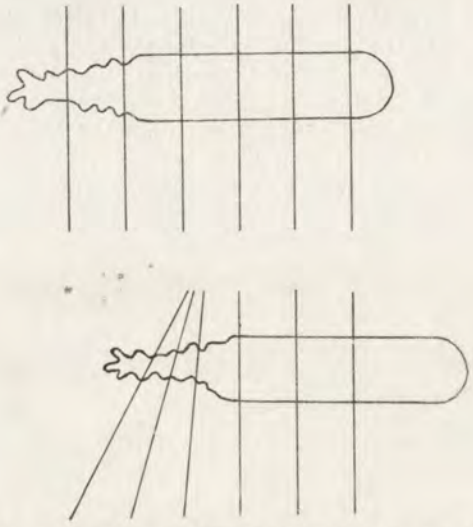

Fig. 4. Resting of the glass fibres put on the surface of a moving amoeba (after Goldacre 1961)

The modern theory of the posterior ectoplasm contraction was recently strongly supported by the study of Rinaldi and Jahn 1963. These authors applied a specific modification of the photographic kimograph for registration of the movements of cytoplasmic inclusions and the photographic registration of the cytoplasmic streaming in the dark field, similar to that used previously for the medium currents around the ciliates (Grębecki 1961) and flagellates (Jahn, Harmon and Landman 1963). Those methods allowed to state that the character of the endoplasm movement inside the advancing pseudopodium is in harmony with the postulations of the tail contraction theory. In the rear of amoeba, as well as in the withdrawing pseudopodia, movements of the plasmagel particles were registered. Their direction was in agreement with that of the sol streaming, or perpendicular to the amoeba axis, as result of the ectoplasm contraction. The most surprising result following from the study of Rinaldi and Jahn 1963 is the concept that the plasmagel is so spongy in structure that it encloses in meshes of its network not only the solution (hyaline liquid) but even a sol-like colloid, i.e. a part of endoplasm is included in clearances of ectoplasm. In effect, the cytoplasmic stream directed from behind towards the anterior part of amoeba, would not be confined exclusively to the axial canal, with no gelated structures, but could flow along its whole body.

Endoplasm contraction in the frontal zone

Although the theory of the posterior ectoplasm contraction in amoeba made recently a great progress, this concept evokes some objections, sometimes essentially justified. Noland 1957 motivates his reserve to accept this theory by the fact that this concept is thoroughly inapplicable — at least in its present form — for explication the rotational cytoplasmic movement (cyclosis) in plant cells, nor even the movement of axopodia and reticulopodia, although both phenomena are probably somewhat connected with the typical amoeboid movement. Even earlier, Swann 1951 put forward an objection that localization of the factor controlling the organism movement in the posterior region of its body seems to be hardly acceptable. Yet the statement of Swann of the eventual motoric factor in the frontal region of amoeba is very unclear.

The fullest criticism of the modern theories of contraction in the posterior ectoplasm was developed by Allen 1961 a and b. The most serious objection put forward by Allen is based on the observations indicating that the phenomena occurring in neighbouring pseudopodia of the same individual are neither correlated nor explicable by the hydrodynamic principles of branching flow and for that reason they could not result from contraction of the common posterior part of the body. Moreover, Allen noticed that the motor response of the front of amoeba to the stimuli is to prompt to be caused by propulsion localized behind.

Allen develops his theory trying to find the motive force of the amoeboid movement in the contraction of the frontal endoplasm of amoeba. This involves changes in the present view on the physical state of endoplasm. Allen and his co-workers really try to prove that the endoplasmic sol of amoeba is not a Newtonian fluid but a pseudoplastic fluid because of its differenced viscosity. The differences in the endoplasm viscosity were studied by observing the behaviour of natural inclusions during centrifuging (Allen 1960) and by sedimentation, in the gravitation field, of gold and iron particles introduced inside the cell (Allen and Griffin 1960).

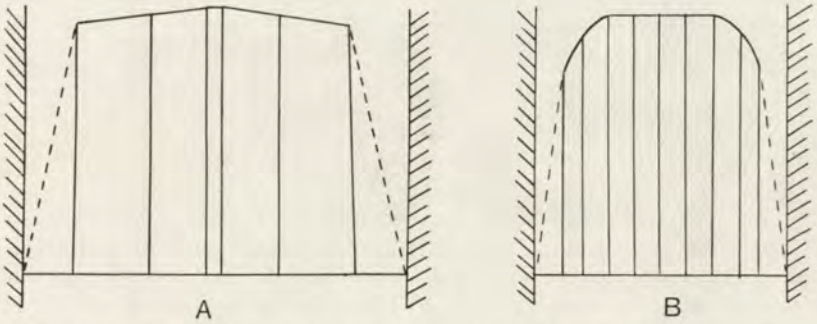

Fig. 5. Velocity profiles of the endoplasmic streaming. Dashed area means ectoplasm, diagrams of velocity being disposed likely inside the ectoplasm tube and arranged conformely to the position occupied in the stream by the particles which velocity was recorded. A. Pseudopodium of *Chaos chaos* (after Allen and Roslansky 1959, modified). B. Plasmodium of the slime mold *Physarum polycephalum* (after Kamiya and Kuroda 1958)

Yagi 1961 — not accepting the theory of Allen — also found the differentiation of the endoplasmic sol viscosity when observing in the magnetic field the movement of nickel particles introduced into amoeba. Similar is the conclusion of Kanno 1964 a.

Especially interesting is the study of Allen and Roslansky 1959 on the velocity profiles of the cytoplasmic streaming inside amoeba. It was proved that only in the posterior part this profile corresponds to the flow of a fluid along a tube, i.e. its velocity, being the highest in the axis of the stream, falls regularly towards periphery. In the anterior part of amoeba these relations are different. The velocity of streaming is nearly uniform on the entire cross section and falls rapidly only on the boundary of sol and gel (Fig. 5 A). It is a plug movement comparable to the ice jam movement on a river or to the movement of a piston inside a cylinder. A similar profil of velocity was found by Kamiya and Kuroda 1958 in plasmodium of slime mold (Fig. 5 B), independently from the above results.

The character of the velocity profiles agrees with the data on the pseudoplastic character of endoplasm. The plug movement would arise then when viscosity increases in a sufficient degree. These results induced Allen 1961 to trace a new scheme of the structure of amoeba (Fig. 6). Besides the elements described before, the axial endoplasm with properties similar to those of the ectoplasmic gel, is presented.

The conviction of the pseudoplastic nature of the axial endoplasm led Allen to the view that it is capable to transmission of directional forces; consequently the contraction may be localized in it[2]. As result, the cytoplas-

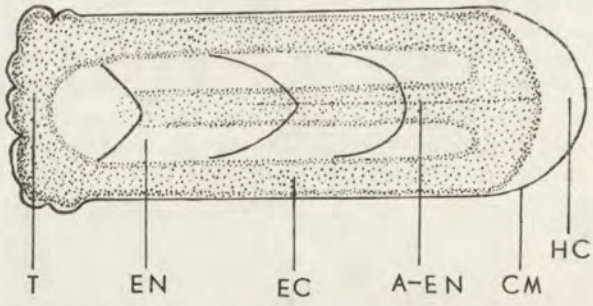

Fig. 6. Scheme of the organization of an amoeba, following Allen 1961 b. EC — ectoplasm, EN — endoplasm, A-EN — axial endoplasm, T — tail region, HC — hyaline cap, CM — cell membrane

mic streaming inside amoeba flows because the axial endoplasm contracts at the head of pseudopodium and sucks the remaining mass of the sol.

Some support of the theory of Allen on the contractility of the pseudoplastic sol are the investigations (Allen, Cooledge and Hall 1960) on the behaviour of the cytoplasm drops kept in a quartz capillary. In their

[2] Somewhat earlier Seifriz 1953 postulated the localization of contractility in the endoplasm itself. He based on the observation of multidirectional movement of neighbouring granulations in cytoplasm and tried to prove a close analogy of moving cytoplasm of different cells and sarcoplasm of the muscle fibres.

interior, intense cytoplasmic streamings are described. The theory of Allen is supported — at last — by his interferometric study of the protein content on both ends of amoeba (Allen and Roslansky 1958) and of localization of the syneresis process (Allen, Cowden and Hall 1962). He also rehabilitates the old description of Schulze 1875, corrected subsequently by Jennings 1904, and stating that sometimes, in the marginal zones of the pseudopodium front, a reverse movement of the cytoplasmic granulations is observed ("the fountain zone"). It is also worth being mentioned that, in opposition to Goldacre 1961, Allen supports the view of Jennings 1904 and of Mast 1926 of the plasmalemma rolling in a moving amoeba (Griffin and Allen 1959).

Active shearing at the sol-gel interface

The third possible variant of the contraction theory of amoeboid movement is the concept of sliding of fibrils, or strictly — of the mutual active shearing of two filaments. This idea originates from the study of the molecular mechanics of the muscular contraction and of the rotational movements of cytoplasm in plant cells. Huxley and Hanson 1954 proved that in contraction of muscle fibres, protofibrils of actin and protofibrils of myosin slide past one another and encroach between one another, producing in this way the threads of actomyosin. Weber 1958 gave a biochemical interpretation of this phenomenon and of the role of ATP in its course.

The concept of active shearing of two filaments is put forward by Kamiya 1959, 1960 a and b, 1961 a and b for explaining the rotatory movement of cytoplasm in cells of *Nitella*. The site of action of the shearing force would be the interface of the ectoplasmic gel and endoplasmic sol. The shearing force should evidently act directionally at a tangent to the revolving endoplasm cylinder. The fundamental arguments in favour of this concept are the velocity profiles of the cytoplasmic streaming in the *Nitella* cells and the microoperations proving that the isolated plasmasol remains motionless but starts streaming in presence of plasmagel.

A similar theory of active shearing was applied by Jahn and Rinaldi 1959 for explanation of the reticulopodia movement in the foraminiferan *Allogromia*. In this case no differentiation of the gel tube with a lumen filled with sol occurs, as in amoebae of proteus type, but the whole reticulopodium seems to be gelated. The cytoplasmic movement is always bidirectional in form of two parallel stripes shifting in opposite directions. Jahn and Rinaldi assume that those stripes consist of two filamentous gel systems, actively sliding one upon another.

Recently an attempt appeared to apply the theory of shearing force to the typical amoeboid movement, i.e. to the cytoplasmic streaming in the slime molds plasmodia. Hayashi 1961 suggests that this streaming is propelled by the active shearing force acting longitudinally on the boundary of the ectoplasmic gel and endoplasmic sol. Evidently the concept of Hayashi would concern amoebae in the same degree as the slime molds.

Other theoretical suggestions

Although the background of the contraction in the cytoplasmic movement has been elucidated, and the dominance of the contractility theories seems to be obvious, there appear still — even in the sixties — some theoretical suggestions trying to explain the amoeboid movement on other principles.

A rather unusual theory of the endoplasm movement in plasmodia of slime molds was developed by Stewart and Stewart 1959a. Basing on the former calculations of Rashevsky 1937 they assume that the cytoplasmic streaming may be evoked by the diffusion drag force. Consequently, streamings would arise as result of different content of various substances in cytoplasm and flow down the concentration gradient.

According to Yagi 1961 a fundamental importance for elucidation the mechanism of amoeboid movement would have the fact, observed by him, that the same portion of amoeba cytoplasm occupies a larger space being in state of sol than in the state of gel. Consequently, the motive force of the cytoplasmic stream would be due to the process of solation itself. Cytoplasm passing from gel-state to sol-state would increase its volume. As a result, the hypertension of sol and its pushing forwards would occur in the only direction in which the rigid ectoplasm cylinder tube is open. The concept of Yagi is in harmony with the theories of the contractility of the posterior part of amoeba in all points except the fundamental detail — omitting the biochemical model of contraction.

The concept developed by T. Abé 1961 is also based upon the sol-gel cycle. T. Abé 1961 is right when stressing that it cannot be assumed that the body of amoeba was deprived of permanent constant structures and that this organism is not fit for morphological examination. Those permanent structures exist but their constituent material is exchangable. So the idea of dynamic morphology or dynamic organization of amoeba may be conceived. The same author in his subsequent article (T. Abé 1962) criticized the contractility theories of the amoeboid movement and — really — all the other concepts. considering the displacement of amoeba as not what biologists use to call movement but rather what they call growth. The most essential moment of the phenomenon under question would be the additive active building of the body in a definite direction. The energetics of this active building is defined by T. Abé only roughly, as resulting from metabolism, and its physico-chemical model mostly corresponds to the sol-gel concepts of the twenties.

The additive active building of the amoeba body forms also the base of the concept of Bell 1961. This author also considers the cytoplasm movement and the contraction activity as not very essential, but he ascribes a much more important role and a more specific localization to the additive active building than it was assumed by T. Abé. In Bell's understanding the displacement of amoeba consists in an intense production of mucoproteins in the front of pseudopodium. As known from the findings of Noland 1957 and Brandt and Pappas 1958 mucoproteins form the external plasmalemma layer. Production of mucoproteins in front of the pseudopodium would involve supplementing of plasmalemma and its active extension. In his later article the same author developed the theory (Bell and Jeon 1962) that the active additive building of plasmalemma is influencing the movement by lowering the surface tension. This conclusion, reflecting the old surface theories (e.g. of Rhumbler 1898), is based on the observation that a local application of heparin evokes simultaneously the protrusion of pseudopodium and fall of the surface tension on the area treated with it.

A quite different meaning of the surface role is developed by Bingley and Thompson 1962. These authors ascertained — using the micro-

electrodes — the presence of a high resting membrane potential in amoebae[3]. More so, they demonstrated that the transmembrane potential difference is always much higher in the posterior of amoeba than in its anterior part; consequently, the front of pseudopodium is distinctly electrically depolarized when compared to the remaining membrane. The general changes in the membrane potential in different ionic media are accompanied by corresponding changes of velocity of the endoplasm stream — and even of its direction in amoeba[4]. The edoplasmic streaming may also be controlled by means of stimulating microelectrodes, applied locally. The local influence of heparin upon the movement and surface tension (Bell and Jeon 1962) is also accounted for by the depolarizing action of this factor (Bingley, Bell and Jeon 1962). Finally, Bingley and Thompson 1962 stated that the cytoplasmic streaming in amoebae is directed towards those regions where the electric membrane depolarization occured. They fail to recognize, however, the amoeboid movement as a simply electrokinetic process but presume that the bioelectric phenomena influence the contraction activeness of amoeba.

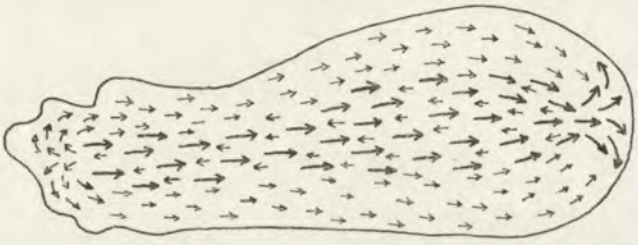

Fig. 7. Turnover of material in the amoeba body according the view of Kavanau. Solid arrows represent the tracks of the self-propelled loose tubular elements in the endoplasm, the fine arrows — the streaming of matrix, pumped forwards by the reticulum tubules in the ectoplasm, and forming a backward countercurrent in the endoplasm (scheme partly based on the drawing of Kavanau 1963 a)

The theory of Kavanau 1963 a is constructed on quite different foundations than the former concepts. The motive force of the amoeboid movement is looked for in the endoplasmic reticulum and especially in the tubular membranous elements. Those tubules, when occurring in ectoplasm, would form a compact, rigid system. Owing to this property they are able to propell the matrix forwards, remaining themselves motionless. But after having

[3] The data concerning the resting potential in amoebae are also known from the studies of Telkes 1931, Buchthal and Peterfi 1937, Wolfson 1943, Umrath 1956, Riddle 1962 and Batueva 1964.

[4] A certain connection between the sign and level of the transmembrane potential difference on one, and the cytoplasmic flow on the other side, is ascertained also in the case of the streaming in the slime mold plasmodia (Kamiya and S. Abe 1950, Tasaki and Kamiya 1950, Diannelidis und Umrath 1952, Kishimoto 1958 a and b) and also in the case of cyclosis in plant cells (Umrath 1953, Kishimoto and Akabori 1959, Vorobev i Vorobeva 1963).

passed to the endoplasmic sol, they become loosely suspended and mobile. For this reason, in the course of pumping the matrix they must flow in the opposite direction, obeing the rule of jet-propulsion. Then, they should flow forwards, pumping the matrix backwards. Finally, in the amoeba body the matrix and the reticulum elements would stream forwards along two different paths: the matrix passively, across the ectoplasm through the body periphery, while the reticulum elements, actively, across the endoplasm near the body axis; both processes should produce together the impression of a general streaming of endoplasm. Really, a part of the invisible matrix would return along the second, backward path (Fig. 7). The concept of Kavanau 1963 a is based on the fact that — despite the failure of the early authors — Danielli 1958 as well as Borysko and Roslansky 1959 found a well developed reticulum in amoeba cytoplasm, by means of electron microscope. Moreover Kavanau tries to document the adequacy of his concept for understanding some peculiar motor phenomena in the locomotion of amoebae (Kavanau 1963 a), as well as for streams in other cells (Kavanau 1963 b).

Emerging paths of synthesis

An attempt to evaluate the current theories

It seems to evoke no doubt that, among different concepts of amoeboid movement, the theory of contraction of the actomyosin-like protein in the posterior ectoplasm has won most adherents. This wiev, developed recently in a most complete form by Goldacre and Lorch 1950, Landau, Zimmerman and Marsland 1954, Goldacre 1961, and Rinaldi and Jahn 1963, explains the majority of the motor phenomena occurring in course of amoeboid locomotion in very consequent and simplest manner. Its postulations are nearly in harmony with the present ideas on the biochemical aspect of movement and on the physical properties of cytoplasm.

Nevertheless, some authors suggest different hypotheses. Many of them catch correctly the essential difficulties met by the concept of the posterior contraction, and suggest a reasonable direction of their solution. Possibly, those hypotheses might rather point out the ways of improving the classical theory instead of becoming foundation for competitive theories which fall in their own new difficulties.

Of course, some views occur which cannot be built into any other theoretical construction. So e.g. the view of Stewart and Stewart 1959 a making the diffusion force responsible for the movement of slime molds, won no adherents. Even if the value of diffusion force was really sufficient to evoke the cytoplasmic streaming — the prompt reactions of the streaming to stimuli would be impossible because the reorganization of concentration gradients requires a considerable period of time. Moreover, a question would remain unanswered: what is the force performing the osmotic work and establishing those gradients?

A separate position occupy also the concepts of T. Abé 1961 and 1962 and of Bell 1961. It seems that introducing of the idea of additive building of the frontal part of amoeba body, instead of that of forward movement, is merely changing the words used, but offers rather little as to the basic explanation. The additive active building is an applicable idea when there

is really no sharp delimitation between the growth of the cell and its movement — as in the case of the neuron (Weiss and Hiscoe 1948) — but not in the case of amoeba. On the other hand, the T. Abé's 1961 term: dynamic organization of amoeba, seems to deserve being applied and the suggestion of Bell 1961 of mucoprotein production in the anterior part of amoeba — is worth being verified as a supplement to the thesis of Goldacre 1961 about restoring the cell membrane at the front of pseudopodium.

The view of Yagi 1961 that the increase of volume in course of solation evokes hypertension in the tail of amoeba, might supplement the theory of the posterior contraction. Yagi himself considers the degradation of ATP to be the source of energy for solation; consequently, introducing the contractile protein into this scheme would be a logical inference from the author's own conclusions. Nevertheless, it remains uncertain whether the observation of the volume increase in the course of solation was correct. It follows from the numerous studies from the laboratory of Marsland (review in Landau, Zimmerman and Marsland 1954) that the cytoplasmic colloids fulfill the criteria of Freundlich 1937 for gels of the second type, i.e. they become solified under the influence of temperature fall or rise of tension, whereas gelation is an endothermic reaction involving increase of volume. As consequence, in course of solation in the tail of amoeba, a loss of volume should occur, instead of its increase. Methodics applied by Yagi 1961 inclines to postulate that this author compared the volume of the normal amoeba with the volume of contracted gel, instead with the volume of the latent gel, as was his intention.

The theory of Kavanau 1963a postulating that the rigid ectoplasm reticulum propels forwards the matrix streaming, and in the endoplasm loose tubular elements flow by themselves, owing to the jet-propulsion (which produces the impression of endoplasm streaming), seems to be hardly acceptable as a whole but might, eventually, supplement the theory of the posterior contraction. The scheme presented by Kavanau for the axial region of amoeba seems little probable, for: 1. the tubular structures of reticulum, as loosely suspended in endoplasm, should move confusely in various directions and would not imitate a uniform streaming; 2. in the cytoplasm streaming, inorganic inclusions with no tubular structure are also streaming forwards, and not backwards, together with the hypothetical counter-current of the matrix. Yet pumping the matrix forwards by the motionless tubular structures of the ectoplasmic gel might seem more plausible. The view of Rinaldi and Jahn 1963 that the stream propelled by the contraction in the posterior body part flows not only in endoplasm but also through the clearances of ectoplasm, could be developed by the thesis that those clearances are not some non-organized meshes of the gel network but the tubular elements of reticulum. This would be in harmony with the general hypothesis of the role of reticulum in transport (Porter and Vamada 1960). Combining the Kavanau's 1963a concept about propelling the matrix along the tubules of reticulum, with the classical contractility theory of the amoeboid movement, would give a general pattern of events in the periphery of amoeba, approached to the present data about the "Nephridialplasma" of the contractile vacuoles in ciliates, where propelling the liquid consists in cooperation of contractile fibrils with the tubu-

lar elements of reticulum (Puytorac 1960 and Schneider 1960). Objections against this logical concept may be evoked by the findings of Wohlfarth-Bottermann and Schneider 1958 and Wohlfarth-Bottermann 1960, that the ectoplasm of plasmodium of slime mold *Didymium nigripes* and of amoeba *Hyalodiscus simplex* is composed of mere matrix with no reticulum elements.

The theory of active shearing of two kinds of filaments formulated by Jahn and Rinaldi 1959 for the movement of reticulopodia of *Allogromia* encounters some difficulties even in this material. Wohlfarth-Bottermann 1961 failed to find two kinds of filaments in *Allogromia* using the electron microscope. The inapplicability of the active shearing theory for explaining the typical amoeboid movement is also realized by its authors themselves. Kamiya 1959, 1960 a and b, 1961 a and b formulated this theory for cyclosis in the plant cells without applying it to slime molds and Jahn and Rinaldi 1959 formulated it for *Foraminifera*, but returned to the tail contraction concept in the case of amoebae (Rinaldi and Jahn 1963).

Possibly, the infirmity of the active shearing concept is the result of too simple direct application of the regularity discovered in the muscle biochemistry on the molecular level (sliding of actin and myosin molecules), to a supramolecular scale (sol-gel interface). Nevertheless, including the sliding mechanism, in its proper molecular meaning, into the theory of the tail contraction, could involve a desirable modernization of this theory. Its largest version (Goldacre and Lorch 1950) is based upon the mechanism of folding and unfolding of the protein molecules, i.e. it corresponds to the state of biochemical research of muscle of, at most, ten years ago when, as mechanism of contraction, the shortening of the actomyosin threads was considered. It might be postulated that the contraction of the posterior part of the ectoplasmic gel in amoeba consists not in folding the molecules of the actomyosin-like protein but in the active shearing of its components — in sliding of the actin-like molecules past the myosin-like ones, up till formation of actomyosin. This modification of the scheme does not distort the important thesis of Goldacre that the concentration performs as well an osmotic work, since formation of an actomyosin complex of its compotents limitates also the free adsorption sites. Just lately, some way of combining the shearing concept with the theory of tail contraction is suggested by Rinaldi 1963.

The most serious difficulty encountered by the theory of the tail contraction is the impossibility to explain the prompt motor response of amoeba to stimulation from forward, if the motive force is localized behind. This is connected with the locomotory autonomy of neighbouring pseudopodia (Allen 1961 a, b). The solution of this problem may be found in the result of Bingley and Thompson 1962 on the electric depolarization of the front of pseudopodium. In contrast to the view of Wohlfarth-Bottermann's 1963 b and 1964 a, including the theses of these authors into the theory of tail contraction presents no essential difficulties, because — really — they are not developing a competitive theory of movement but a theory of excitation.

Depolarization of the anterior part of the cell membrane produces fundamental excitation events, as in ciliates and even in the excitable tissue

cells; thereafter the excitation sets in motion the specific contractile apparatus. If that was possible, either the conduction of the impulse to the posterior — permanently contracting part of amoeba — is necessary, or interference of another, supplementary, contraction in the anterior region, which would strengthen or reduce the cytoplasmic streaming, or lead it to a new direction. Conducting impulses in amoeba which might transmit the information backwards, determining not only the velocity but also the direction of the streaming, is unknown. It is also difficult to imagine how the posterior parts could influence the streaming direction at the front of the pseudopodium. Consequently, the existence of another, anterior, contraction centre seems more probable and in this meaning the tail contraction theory might be supplemented in the direction postulated by Allen 1962 a and b. However, only a supplementary steering role might be ascribed to the second contraction centre and the main source of the motive force should not necessarily be looked for in it, as is done by Allen. Moreover, a contraction giving direction and regulating the velocity, might do not cooperate in locomotion (being an endoplasm contraction — according the theory of Allen 1961) but it may modify the direction and velocity by inhibiting it. So it would be a counter-contraction of the anterior part of the ectoplasmic tube.

Crucial points of research

All the modern theories of the amoeboid movement are highly hypothetic despite the great progress achieved in the last ten years in this field. Nothing more than a working hypothesis may be the suggestion discussed above, concerning the inclusion of the most valuable concepts taken from different, competitive theories, into the theory of tail contraction. Many problems need being intensively studied before we know about the amoeboid locomotion at least as much as we already know about other biological forms of movement.

As to the contractile apparatus of amoebae, we only know presently that it involves an actomyosin-like protein. It was first separated from slime molds and only recently also from amoebae. In both cases, too little is known about its structure and its properties. It seems necessary to prove whether it is composed — like in mucles — of components of the actin and myosin type, and whether its reaction with ATP results in forming an actomyosin complex by sliding the molecules past one another. Especially important seems to be the study of the role of the Ca ions in the amoeba activity, because it could be expected that here Ca — like in the muscle — is the excitation-contraction coupling agent.

The rather scarce data about the contractile apparatus supplied by the electron miscroscope study were very disappointing for a long time. Also the informations about the degree of development of the cytoplasmic reticulum in amoebae were controversive. All that inclines to conclusion that those structures are very unstable and disintegrate easily in fixation (cf. Wohlfarth-Bottermann 1963 b, 1964 a). A critical review of data obtained by different ways is necessary as well as searching for new more discerning methods. As to the contractile apparatus — as well as to the reticulum — further electron microscope study of amoebae, induced experimentally to a high degree of gelation or solation, seems desirable. Investigation of this

kind have been recently initiated by Wohlfarth-Bottermann 1962, who found contractile fibres in drops of slime mold endoplasm only a certain time after they had been isolated from the interior of plasmodium and just partly gelated.

Among the problems of movement mechanics, the diversity of opinions concerning the real existence of the rolling movement of plasmalemma, as reported by Jennings 1904, seems to be puzzling. The concept of Goldacre 1961 about the production of new membrane at front of pseudopodium and its resorption at the tail of amoeba seems more justified and its experimental support more convincing. More so, concept of the membrane reproduction opens some very interesting possibilities of synthesis, initiated lately by Wohlfarth-Bottermann 1963 b, 1964 a, relating the behaviour of the membrane in the course of the amoeboid movement and the membrane flow in pinocytosis (Bennett 1956). However, presently the membrane rolling has been described again by T. Abé 1961 and by Griffin and Allen 1958. It is very possible that the movement of foreign particles along the plasmalemma surface, is not connected with its own movement in amoebae of the proteus type. Main reports on the rolling movement are concerning the monopodial amoebae with a distinctly differentiated surface as just *Thecamoeba verrucosa* (Jennings 1904) and *Thecamoeba striata* (T. Abé 1961) are. At any rate, even for purely geometrical reasons, it can hardly be imagined how the rolling of the membrane would occur in all directions and dimensions, in the polypodial amoebae.

The studies of velocity profiles, executed in the laboratory of Allen, seem to decide the matter of the high and differenced viscosity in the endoplasm streaming. Yet it would be desirable to develop them applying the most objective method of photographic registration of the cytoplasmic streamings[5]. It ought to be realized however, that — in contrast to the opinion of many authors — the analysis of the velocity profiles cannot decide about the validity of the posterior or the anterior contraction theory nor about the active shearing. The profiles prove merely that endoplasm, gaining a considerable viscosity, starts moving like a piston in a cylinder. The piston, however, moves in the same manner, either when pushed from behind, or drawn from forward, or actively sliding along the walls of the cylinder.

Decisive for the theory should be the solution whether the endoplasmic sol, produced from the cell, would be contractile. The investigations should be resumed because Allen, Cooledge and Hall 1960 described movement in isolated endoplasm drops, and Goldacre 1961 denies this fact. An essential indication in this problem might be the results from the study of plant cells. Kamiya 1960, 1961 a and b maintains that the drops of pure endoplasmic sol of the *Nitella* cells remain motionless, and the cytoplasmic streaming appears only in presence of a gel phase in them. In the endoplasm drops sucked out of the *Chara* cells, the condition of movement is also the presence of both phases: it appears only in the form of chloroplasts rotation (Jarosch 1956) or of nuclei (Fetzmann 1958). Thus, only the observation of eventual cytoplasmic streaming in the endoplasm drops of amoebae after having ensured their entire solation and having made impos-

[5] Such an investigation was done just recently by Rinaldi 1963, entirely confirming the non-Newtonian character of the flowing endoplasm.

sible a partial gelation — might give some essential support to the concept of Allen 1961, that the contraction can be localized in the endoplasm itself as long as it still remains endoplasm.

The mechanism of response of amoeba to stimuli, acting at the front of pseudopodium, requires the most intensive investigations, being the most serious difficulty of the tail contraction theory. The feed-back down the hyaline layer — as suggested by Goldacre 1956, 1957, 1961 — explains rather the facultative stability of the cytoplasm streaming, not its changes introduced by the stimulation. Nearly all the data about the action of the ionic medium upon the amoeboid movement originate from the first tenths of the present century. The experiments were, then, schemed with ignorance of this ionic background of excitation and response, which is known at present time. Our knowledge about the behaviour of amoebae, under the influence of electric stimulation, remains also at the level which was achieved in the study of galvanotaxis of ciliates, already in the time of Jennings. It may be expected that a more accurate analysis of the behaviour of amoebae under the influence of chemical and electric stimuli would allow to prove whether another contraction centre in the anterior part of amoeba really exists, and whether its steering action is based on the priciple of an inhibiting counter-contraction. Evidently, the analysis of the responses to stimuli should be supported by the study of electrophysiological parameters of excitation in amoebae, as just recently initiated.

Summary

Recent biochemical and electron microscope data are summarized, which reveal the submorphological background of the motor activity in the amoeboid locomotion and some related forms of the cellular movement. The three main current theories are presented — the concept of a posterior ectoplasm contraction, of an anterior endoplasm contraction, and the active shearing hypothesis — as well as some other concepts postponing the contractility mechanism. The view is developed in details that the majority of valuable assumptions of the competitive theories lead not obviously to the rejection of the concept of a posterior ectoplasm contraction but — on the contrary — they might, without esential difficulties, corroborate in the improvement of this classical and the most viable theory.

STRESZCZENIE

Przytoczono w skrócie współczesne dane biochemii i mikroskopii elektronowej, które ujawniają submorfologiczne podłoże aktywności motorycznej w trakcie ruchu amebowego i pewnych związanych z nim form ruchu w komórce. Przedstawiono trzy najważniejsze bieżące teorie — koncepcję tylnego skurczu ektoplazmy, przedniego skurczu endoplazmy, oraz hipotezę czynnego poślizgu — a także niektóre inne koncepcje pomijające mechanizm skurczowy. Szczegółowo rozwinięto pogląd, że większość cennych tez teorii konkurencyjnych nie musi koniecznie prowadzić do odrzucania koncepcji tylnego skurczu ektoplazmy, lecz przeciwnie — mogą one bez zasadniczych trudności pomóc w ulepszaniu tej klasycznej i najżywotniejszej teorii.

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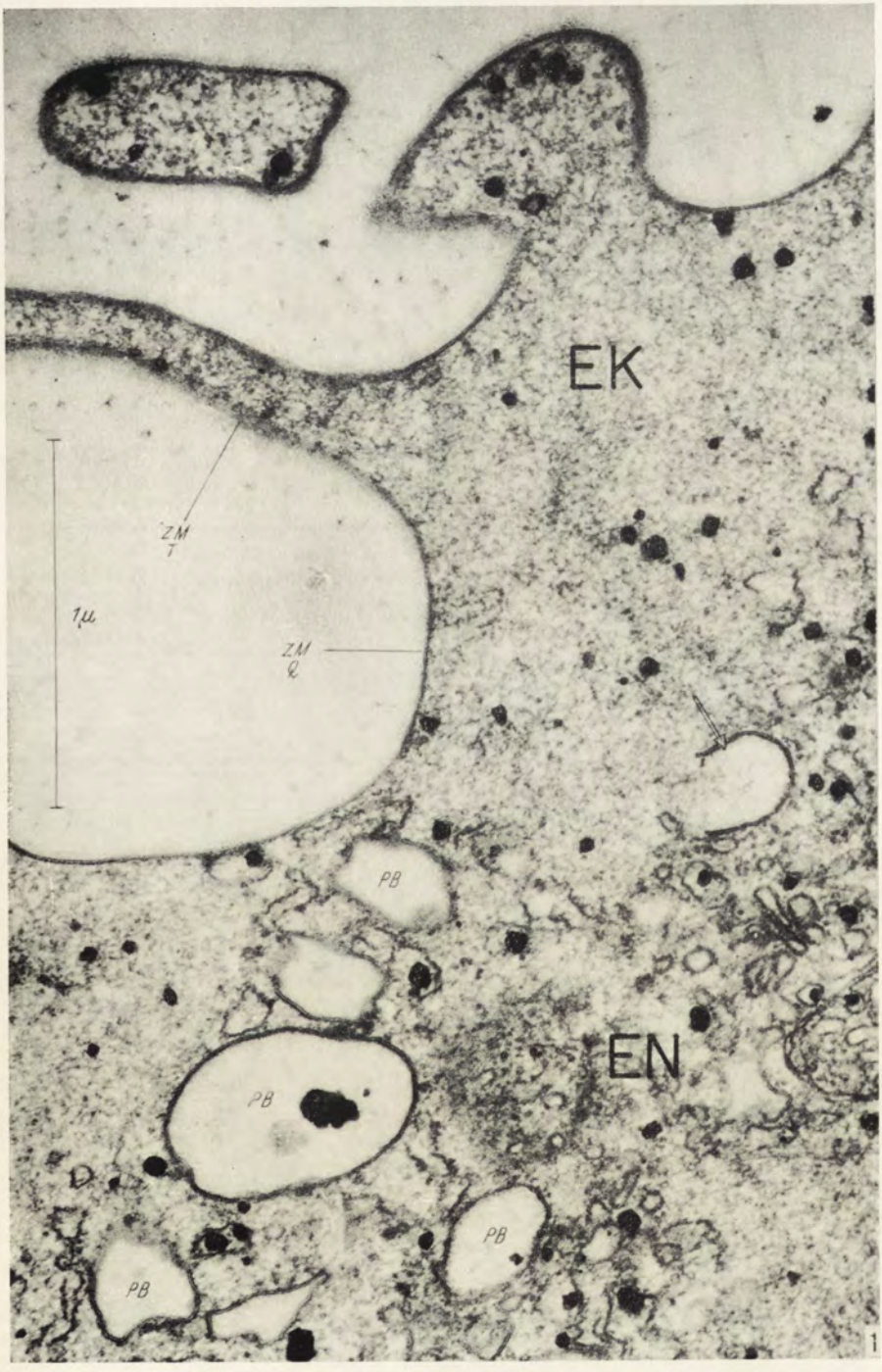
EXPLANATION OF PLATES I—II

1: Differences of the fine structure of ectoplasm and endoplasm in the amoeba *Hyalodiscus simplex*. Note the presence of a well developed vesicular structure of the endoplasm.

2: Filaments in the ectoplasmic region of cytoplasm in the amoeba *Hyalodiscus simplex*.

3: Filamentous structures in a fragment of plasmodium of the slime mold *Physarum polycephalum*.

[Symbols: EK — ectoplasm, EN — endoplasm, F — filamentous structures, PB — pinocytotic vacuoles, ZMQ — cross section of the cell membrane, ZMT — its tangential section. All the electron micrographs were performed in the Zentrallaboratorium für angewandte Übermikroskopie der Universität Bonn]



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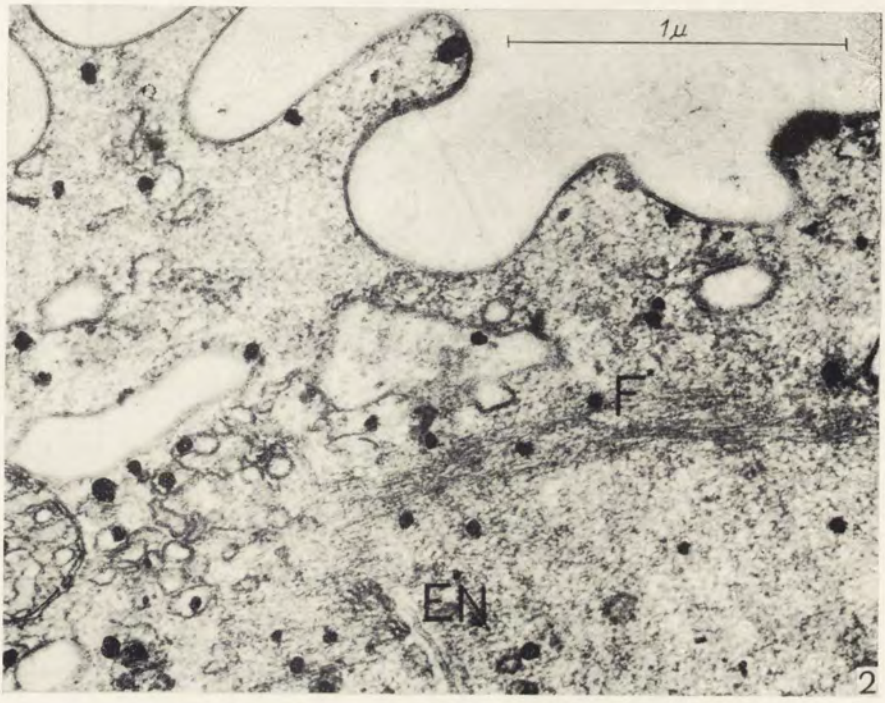

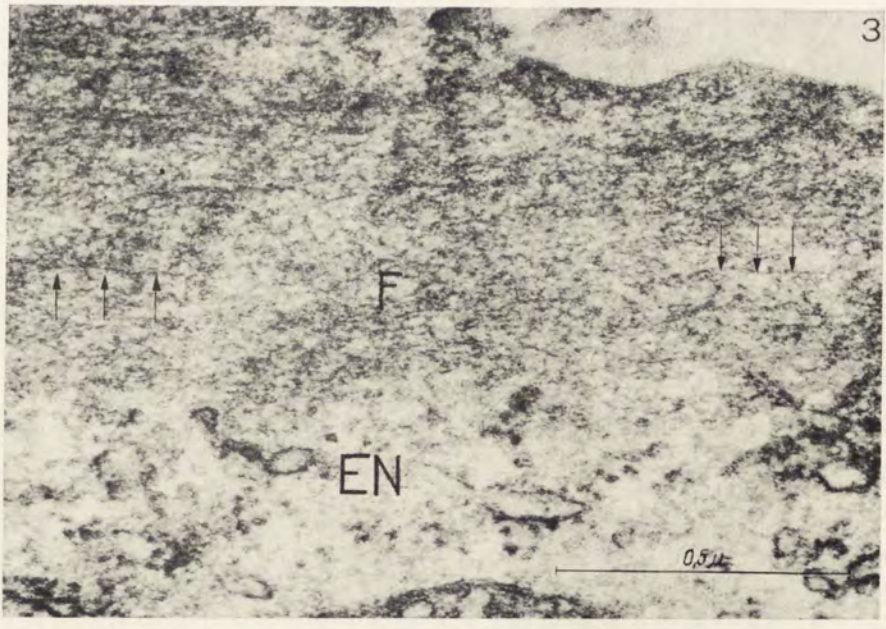

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