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ACTA PROTOZOOLOGICA
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Zoological Survey of India, 8, Lindsay Street, Calcutta-16, India

A. K. MANDAL

Trypanosoma choudhuryi sp. nov. from *Tilapia mossambica* (Peters)

Synopsis. A new trypanosome, *Trypanosoma choudhuryi* sp. nov. is described from a fresh water fish, *Tilapia mossambica* (Peters). It is monomorphic and atenuated at both ends, measuring 13.25 μm in total length. Volutin granules present in the cytoplasm and mainly localized at the border opposite to the undulating membrane. Its affinities with the allied species have been discussed and incorporated in this paper.

This is the third instalment of the series deals with the trypanosomes of Indian fishes. In the earlier issues, Mandal (1975, 1976) described four species of trypanosomes from Indian fishes of commercial importance.

The present communication represents a new species of trypanosome obtained from ten examples of *Tilapia* out of 60 examples examined during the year 1974–1975. *Tilapia* being an exotic fish has become commercially important and occupied a prominent place in the fish markets of Calcutta. So far, only three species of trypanosomes described from *Tilapia* by Travassors Santos Dias (1955) from East Africa. The present report deals with a new species of trypanosomes from *Tilapia* from Indian subregion.

Material and Methods

The fishes were procured from the different markets of Calcutta and kept in the laboratory for examination. The blood was generally taken after puncturing the branchial blood vessels. Wright, Leishman and Giemsa's stains were used for staining. Some organ smears have been prepared and the tissues, like kidney, lungs, brain were fixed. The sections were cut and examined for the endogenous stages of the parasite. Some leeches viz. *Hemiclipsis* sp. were also procured from the suburb of Calcutta or from the same habitat of the fishes examined. After being kept them at starvation for 2/3 months in the Laboratory, the leeches were allowed to feed on the infected fishes at the Labo-

ratory and examined at certain intervals. Cross sections after fixation were also made for the endogenous development. But except in the peripheral blood of the host nowhere the trypanosomes were possible to trace. The figures were drawn with the help of Camera Lucida and measurements were taken by placing a thread along the middle of the parasite. Five to thirty trypanosomes were counted in a parasite film. The type specimens will be deposited to the National Zoological collection, Zoological Survey of India.

Observation

Trypanosoma choudhuryi sp. nov.

Figs. (1–4)

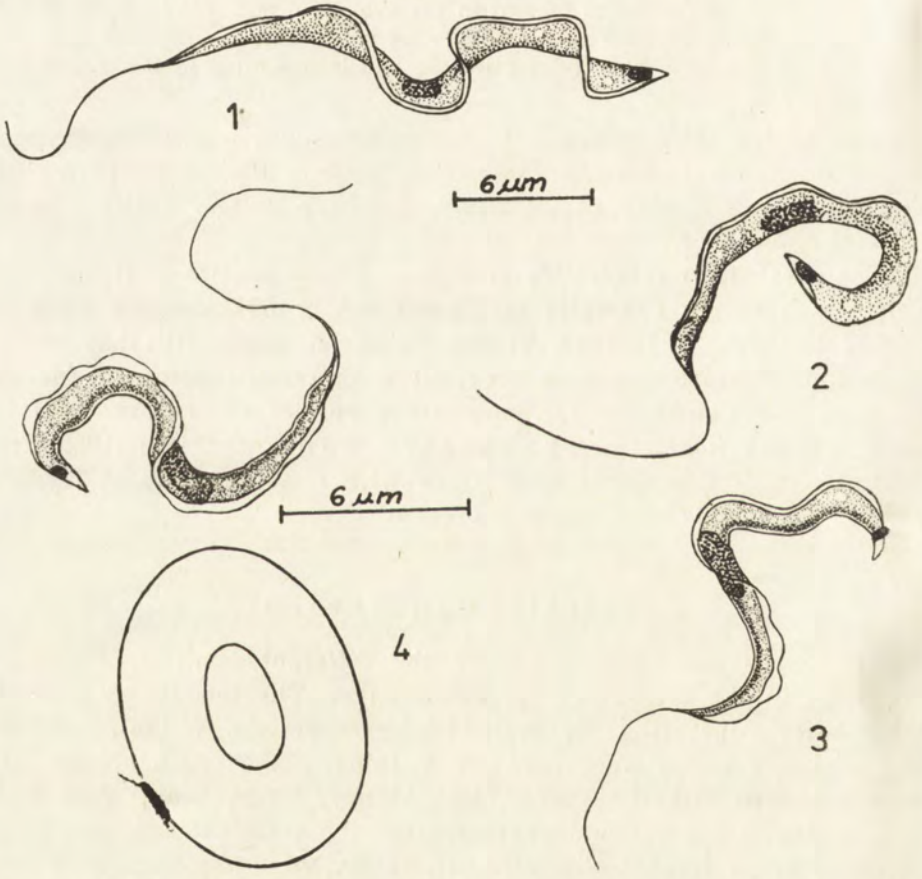

Fig. 1. *1* — *Trypanosoma choudhuryi* sp. nov. showing concentration of granules at one side, *2–4* — *T. choudhuryi* sp. nov. in the forms of 'C' and 'S'.
Figs. 1–3 — at same magnification, Fig. 4 — trypanosome and erythrocyte of *Tilapia* in the other magnification.

Description: The trypanosome is monomorphic elongated and atenuated at both ends. Their configuration varies from a 'C' to an 'S' (Fig. *2–4*). No polymorphism was noted and no divisional stages were found in the blood or any organ smear preparation. Length of the cell body 20.23 μm (range 16.5–25.32 μm); length of the free flagellum 10.79 μm (range 6.5–12.5 μm); total length 31.25 μm; distance from anterior end of the body to the anterior end of the nucleus 6.64 μm (4.5–8.5 μm), length of the nucleus 4.15 μm (range 3.5–5.25 μm); width of the nucleus 1.24 μm (range 1.0–2.5 μm), distance from posterior end of the nucleus to the kinetoplast 7.4 μm (range 5.5–9.5 μm); length of the kinetoplast 1.2 μm (range 1 to 1.57 μm); width of the kinetoplast 0.58 μm (range 1–1.05); distance from kinetoplast to the posterior tip 1.5 μm (range 0.8–2.5 μm); width of the undulating membrane 0.5 μm (range 0.4–0.85 μm); width of the cell body 1.5–1.8 μm.

Cytoplasm: The cytoplasm stains light blue. Sometimes two large vacuoles are present at both extremities of the nucleus. Numerous granules are present in the cytoplasm, generally localized at the border opposite to the undulating membrane and in most instances these granules are concentrated more towards the portion anterior to the nucleus.

Nucleus: The nucleus is sausage-shaped situated almost at the middle of the body proper, stains deep blue and not occupying the entire width of the body. In most specimens there appear one dark blue stain karyosome in each.

Kinetoplast: The kinetoplast is generally oval, but in many occasions it is found conical; staining very deep, and sometimes appearing as deep-blue body. It is always large in relation to the width of the posterior end of the body where it is situated.

Flagellum and undulating membrane: The flagellum originates from the kinetoplast and trails anteriorly bordering the undulating membrane and extending beyond the body as a free flagellum. The undulating membrane stains light blue and is clearly outlined with 4–6 folds.

Type host: *Tilapia mossambica* (Peters)
Site of Infection: Blood
Type Specimens: Holotype Reg. Pt 1849
Paratype Reg. Pt 1850, Pt 1851, Pt 1852
Locality — Bagmari, 24 Parganas, W. Bengal
Vector and life cycle — Unknown.

Diagnosis of *Trypanosoma choudhuryi* sp. nov.

The trypanosome is monomorphic, measuring 31.25 μm in total length. Numerous volutin granules are present in the cytoplasm mainly localized at the border opposite to the undulating membrane. Nucleus

sausage-shaped situated almost at the middle of the body and does not cover the entire width of the body. Kinetoplast is always large in relation to the width of the posterior end of the body where it is situated Undulating membrane distinct and the number of folds varies from 4–6.

Remarks

The present species is monomorphic and has got some resemblances with *T. danilewski* Laveran et Mesnil, 1904 and *T. danilewski saccobranchii* Qadri, 1952 due to shape and size but the former is smaller in length of the cell body proper as well as in the free-flagellar length. Moreover, the present species does not resemble to three species viz. *T. napolesi, T. rebeloi* and *T. serrenoi* already described from *Tilapia* by Travassos Santos Dias (1955) or any of the known species.

Therefore, the present form is described as new and named as *T. choudhuryi.* The species name is given after Dr. Amalesh Choudhury an eminent Protozoologist.

ACKNOWLEDGEMENTS

Thanks are due to Dr. S. Khera, Deputy Director in charge and Dr. T. D. Soota, Superintending Zoologist, Zoological Survey of India for providing me the facilities in carrying out this work.

RÉSUMÉ

Une nouvelle espèce, *Trypanosoma choudhuryi,* est décrite d'un poisson, *Telapia mossambica* (Peters), qui vit dars l'eau douce. Elle est monomorphique et atténuée à toutes ses deux extrémités, ayant 13.25 μm de long jusqu'au bout. Le cytoplasme est muni des granules volutines localisées principalement au bord en face de la membrane onduleuse. La discussion sur les affinités avec ses espèces proches est aussi inclue dans cet article-ci.

REFERENCES

Laveran A. and Mesnil F. 1904: Trypanosomes et trypanosomiases. Paris, pp. XI + 417.

Mandal A. K. 1975: Two new trypanosomes from Indian fresh water fishes. Angew. Parasitol., 16, 87–93.

Mandal A. K. 1976: Two new species of trypanosomes (*Protozoa: Mastigophora*) from Indian fresh water fishes. II. Angew. Parasitol. (in press).

Qadri S. S. 1952: On three new trypanosomes from fresh water fishes. Parasitology, 52, 221–228.

Travossos Santos Dias J. A. 1955: Estudos sobre os hematozoarios dos peixes de aqua doce de Mocambique. I. Descsicao de algumas novas espécies de tripanossomas parasites da *Tilapia mossambica* (Peters, 1852). Mocambique, 82, 47–63.

ACTA PROTOZOOLOGICA

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Paul Josef DEDERICHS and Erich SCHOLTYSECK[1]

New Gregarines of the Genus *Gigaductus* from *Carabidae*

Synopsis. Two new species of gregarines of the genus *Gigaductus* are described from carabids: *Gigaductus agoni* n. sp. from *Agonum sexpunctatum* is characterized by a maximum gamont length (66 μm), diameter of gamontocysts (42–49 μm), and size of sporocysts (11.7×6.5 μm). Indications of a schizogony are present. *Gigaductus brachyni* n. sp. from *Brachynus crepitans* is characterized by gamont length (58 μm), shape of gamonts and occurrence of prominent dark granules in the gamont cytoplasm. Further stages of the life cycle of this species are yet unknown.

Gregarines of the genus *Gigaductus* have been the subject of special interest for some years. In 1948 Filipponi assumed that the early development of *Gigaductus exiguus* takes place within the host cell (cf. Geus 1969). This way of development, although very atypical of cephaline gregarines was confirmed by Tuzet and Ormieres (1966) light-microscopically for *Gigaductus anchi* from *Anchus ruficornis*. Electrone microscope studies by Ormieres (1971) on the same species revealed in addition that this intracellular development includes an asexual multiplication by schizogony. For the other species of the genus *Gigaductus* no indications of schizogony or intracellular development are reported up to now. Further investigations on gregarines of this genus seemed to be of interest.

Materials and Methods

Specimens of *Agonum sexpunctatum* (*Carabidae*) were obtained in the Kottenforst near Bonn/W.-Germany, those of *Brachynus crepitans* (*Carabidae*) were found at Brodarica near Sibenik/Yugoslavia. All investigations were made 2 to 3 hours after the beetles were caught. The beetles were killed by decapitation and an incision was made near the last abdominal segment. By this method it was possible to extract the gut as a whole. The complete gut was surveyed in order

[1] Supported by the Deutsche Forschungsgemeinschaft.

to locate the infected parts. For closer examination the gut was repeatedly sectioned. All investigations were carried out on living material. Gamontocysts were allowed to sporulate in damp chambers which were prepared as follows: the bottom of a Petri dish was covered with damp absorbent paper, and a slide with gamontocysts was placed inside it. Pieces of crushed glass were used to elevate the slide above the damp paper. The chamber was sealed hermetically by replacing the lid of the Petri dish with some drops of water resulting in the formation of a thin layer of water between the lid and the base. All measurements were made with an ocular micrometer at a magnification of 400 × and are given in µm with the ranges in parenthesis. For biometric analysis the following abbreviations are used:

TL:	total length of gamont	WP:	width of protomerite
LP:	length of protomerite	WD:	width of deutomerite
LD:	length of deutomerite	N:	nucleus

Results

1. *Agonum sexpunctatum*

All beetles of this species showed a heavy infection with gregarines; about 2000 to 3000 gamonts and some gamontocysts were found in each host. The infection was concentrated on the gut crypts, although the other parts of the gut were also infected. Heavy hypertrophy of gut cells was observed especially in the crypts.

The gamonts (Pl. I 1.2) of the gregarines from *Agonum sexpunctatum* showed the following characteristics: their protomerites had a more or less circular shape and were always wider than high. The septum between protomerite and deutomerite was level or little depressed towards the deutomerite. The deutomerites were rotund or oval. The spherical nucleus was located somewhat behind the middle of the deutomerite and contained a prominent, dark nucleolus (Pl. I 3). Older gamonts were filled quite densely with rather coarse-grained reserve material. Among the gamonts binucleated stages were found in some cases (Pl. I 3). Epimerites and syzygies were not observed.

Biometric analysis of gamonts:

50 gamonts had the following average measurements:

TL:	43 (28–66) µm	WP:	19 (13–29) µm
LP:	13 (7–21) µm	WD:	28 (20–45) µm
LD:	30 (19–46) µm	N:	9 (8–10) µm

The gamontocysts sporulated in the course of 1–2 days in a damp chamber. In newly encysted gamontocysts the two gamonts were still recognizable (Pl. I 4); older gamontocysts appeared as a single dark stained mass (Pl. I 5). In sporulated gamontocysts the sporocysts were

perceived through the wall of the gamontocyst. A thick outer layer (OL) was present around the periphery of the gamontocyst (Pl. I 6 a). Each gamontocyst had about 40 to 50 sporocysts which were evacuated by one long sporoduct. The sporocysts were relatively large and cylindrical (Pl. I 6 b). 15 gamontocysts measured 45 (42–49) µm and 50 sporocysts 11.7 (10.6–13) × 6.5 (6–7) µm.

2. *Brachynus crepitans*

A *Brachynus crepitans* caught near Sibenik/Yugoslavia was infected with 14 gamonts of gregarines. All beetles of this species obtained in Germany were free of gregarines.

The protomerites of the gamonts were circular or oval. The shapes of the deutomerites, on the other hand, varied, and only one feature was common to them all: the maximal width was reached in the posterior part near the end. Deutomerites were symmetrically shaped and more or less oval (Pl. II 7,8), curved (Pl. II 9), and irregularly contoured (Pl. II 10, 11). All gamonts were filled with a pale granular material, so that they appeared almost translucent with light microscopy. However, in all gamonts a number of prominent, dark granules were seen (Pl. II 8).

Biometric analysis of 14 gamonts

TL:	48 (42–56) µm	WP	17 (15–19) µm
LP:	14 (11–15) µm	WD:	20 (17–23) µm
LD:	34 (28–41) µm	N:	7.5 µm

Discussion

1. *Agonum sexpunctatum*

No gregarines have been previously described from *Agonum sexpunctatum*. Based on the following criteria the gregarine species from this beetle may be placed into the genus *Gigaductus* Crawley, 1903:

(a) the total length of the gamonts is, as in all *Gigaductus*-species, comparatively low (maximum of the genus: 150 µm).

(b) the shape of the gamonts resembles the shape of *Gigaductus*-species already described (cf. Geus 1969, and Tuzet and Ormieres 1966).

(c) the gamontocysts sporulate in a short time (1–2 days) and contain comparatively few, large sporocysts which are evacuated by one long sporoduct.

(d) in spite of the low number of sporocysts per gamontocyst (40–50) the infection is very heavy in every case; this is suggestive of schizogony.

Further indications of schizogony, as described for *Gigaductus anchi* by Ormieres (1971), are the presence of binuclear stages and signs of intracellular development.

(e) no epimerites have been found by light microscopy in the present study nor in the majority of other descriptions of *Gigaductus*-species. An exception is the description of an epimerite by light microscope for *Gigaductus podurae*, a gregarine whose systematic position, in our opinion, seems to be doubtful (cf. Geus 1969). Ormieres (1971), on the other hand, showed that *Gigaductus anchi* possesses an epimerite which can be seen only by electron microscope.

From *Carabidae* five species of *Gigaductus* are known. These have the following biometric data (cf. Tuzet and Ormieres 1966):

species	gamonts	gamontocysts	sporocysts
G. parvus	150 µm	120–200 µm	25×10 µm
G. macrospora	142.5 µm	55–115 µm	20×8 µm
G. elongatus	105.6 µm	58–83 µm	14.16×6.7 µm
G. anchi	85 µm	45–56 µm	16×6.5 µm
G. exiguus	75 µm	45–55 µm	11.6×4.7 µm

The species from *Agonum sexpunctatum* has the measurements:

66 µm 42–49 µm 11.7 × 6.5 µm

Comprehensively, the *Gigaductus* species from *Agonum sexpunctatum* shows the following differences to the species already described:

(a) the maximum total length of the gamonts is distinctly shorter than the maximum length of the species previously described.

(b) the diameter of the gamontocysts is narrower than that of other species described.

(c) the measurements of the sporocysts in the present study differ from those species with similar sporocyst sizes, clearly, at least in one respect: by length from *G. elongatus* and *G. anchi*, by width from *G. exiguus*.

Our results indicate that the *Gigaductus*-species from *Agonum sexpunctatum* is not identical to those already described. Therefore this species is named *Gigaductus agoni* n. sp.

2. *Brachynus crepitans*

The classification of the gregarines from this beetle is difficult because only gamonts were found but no epimerites, gamontocysts or sporocysts. Characteristics of these gamonts, however, indicate again a placement into the genus *Gigaductus*:

(a) the shape of the gamonts is similar to other *Gigaductus* species.

(b) the low total length of the gamonts (60 µm). This character is typical of the genus *Gigaductus*.

(c) the fact that no epimerites were seen by light microscopy, although this cannot be regarded proof in view of the low number of gamonts gound.

Several additional characteristics indicate that these gregarines belong to a species not yet described:

(a) the length of the gamonts remains considerably shorter than the maximum length of the smallest *Gigaductus*-species known before (*G. anchi* 85 µm, *G. agoni* n. sp. 66 µm).

(b) no similar variation in the shape of the deutomerites was described for any *Gigaductus*-species before.

(c) none of the known *Gigaductus*-species showed the prominent granules as described.

This species is named *Gigaductus brachyni* n. sp.

ZUSAMMENFASSUNG

Zwei neue Gregarinenarten der Gattung *Gigaductus* werden aus Carabiden beschrieben. *Gigaductus agoni* n. sp. aus *Agonus sexpunctatum* ist charakterisiert durch das Maximum der Gamontenlänge (70 µm), die Größe der Gamontocysten (42–49 µm) und die Größe der Sporocysten (11.7 × 6,5 µm). Anzeichen einer Schizogonie wurden beobachtet. *Gigaductus brachyni* n. sp. aus *Brachynus crepitans* ist gekennzeichnet durch Gamontengröße (60 µm), Gamontenform und Vorkommen von großen, dunklen Reservestoffkörnern in den Gamonten. Die weiteren Stadien des Entwicklungsganges dieser Art sind noch unbekannt.

REFERENCES

Geus A. 1969: Sporentierchen, *Sporozoa*. Die *Gregarinida* der land- und süßwasserbewohnenden Arthropoden Mitteleuropas. In: Die Tierwelt Deutschlands und der angrenzenden Meeresteile nach ihren Merkmalen und nach ihrer Lebensweise (ed. Dahl, F.). Jena. pp. 608.

Ormieres R. 1971: Une gregarine paradoxale, *Gigaductus anchi* Tuz. Orm., 1966: Ultrastructure des stades schizogoniques et position systematique des *Gigaductidae* Filipponi, 1948. Protistologica, 7, 261–271.

Tuzet O. and Ormieres, R. 1966: *Gigaductus anchi* n. sp., gregarine parasite d'*Anchus ruficornis* Goeze (*Coleoptera, Caraboidea*) et le problème des *Gigaductidae*. Protistologica, 2, 43–49.

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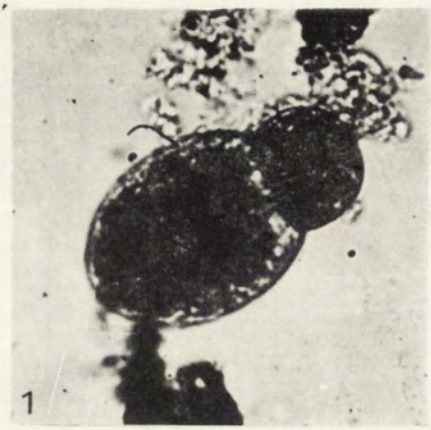
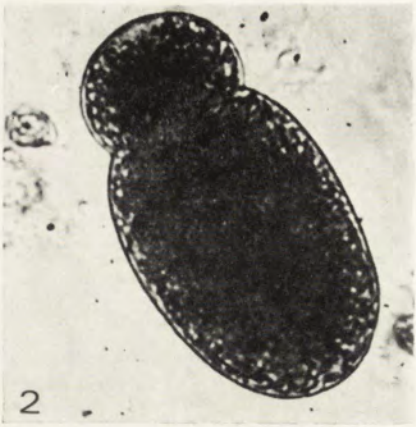
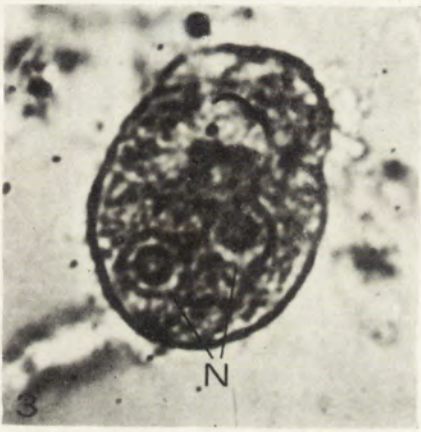
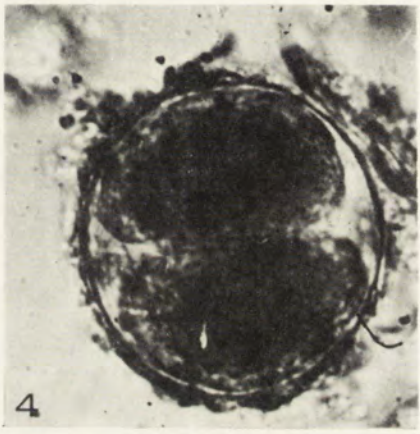
EXPLANATION OF PLATES I–II

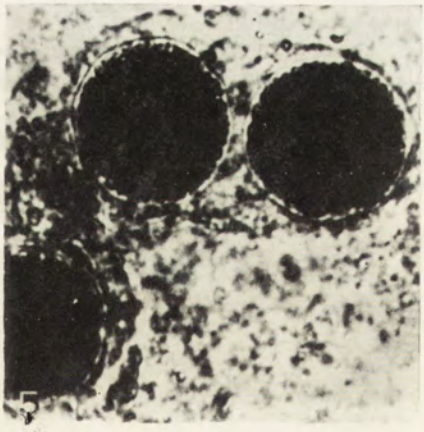
1,2: Gamonts of *Gigaductus agoni* n. sp. 1: 1000 ×. 2: 1200 ×.
3: Binucleated stage of *Gigaductus agoni* n. sp. N: nucleus. 1400 ×.
4: Newly encysted gamontocyst of *Gigaductus agoni* n. sp., the two gamonts still ll recognizable. 1100 ×.
5: Older gamontocysts of *Gigaductus agoni* n. sp. 600 ×.
6a: Sporulated gamontocyst of *Gigaductus agoni* n. sp. Sporocysts (S) visible through h gamontocyst wall. Outer layer around the periphery of the gamontocyst. 1100 ×.
6b: Sporocyst of *Gigaductus agoni* n. sp. 1000 ×.
7–11: Gamonts of *Gigaductus brachyni* n. sp. 7: 1100 +.
8: GR: granules. 1000 ×. 9: 1100 ×. 10: 1150 ×. 11: 1150 ×.

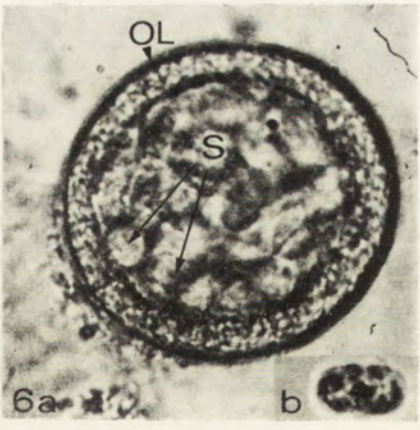












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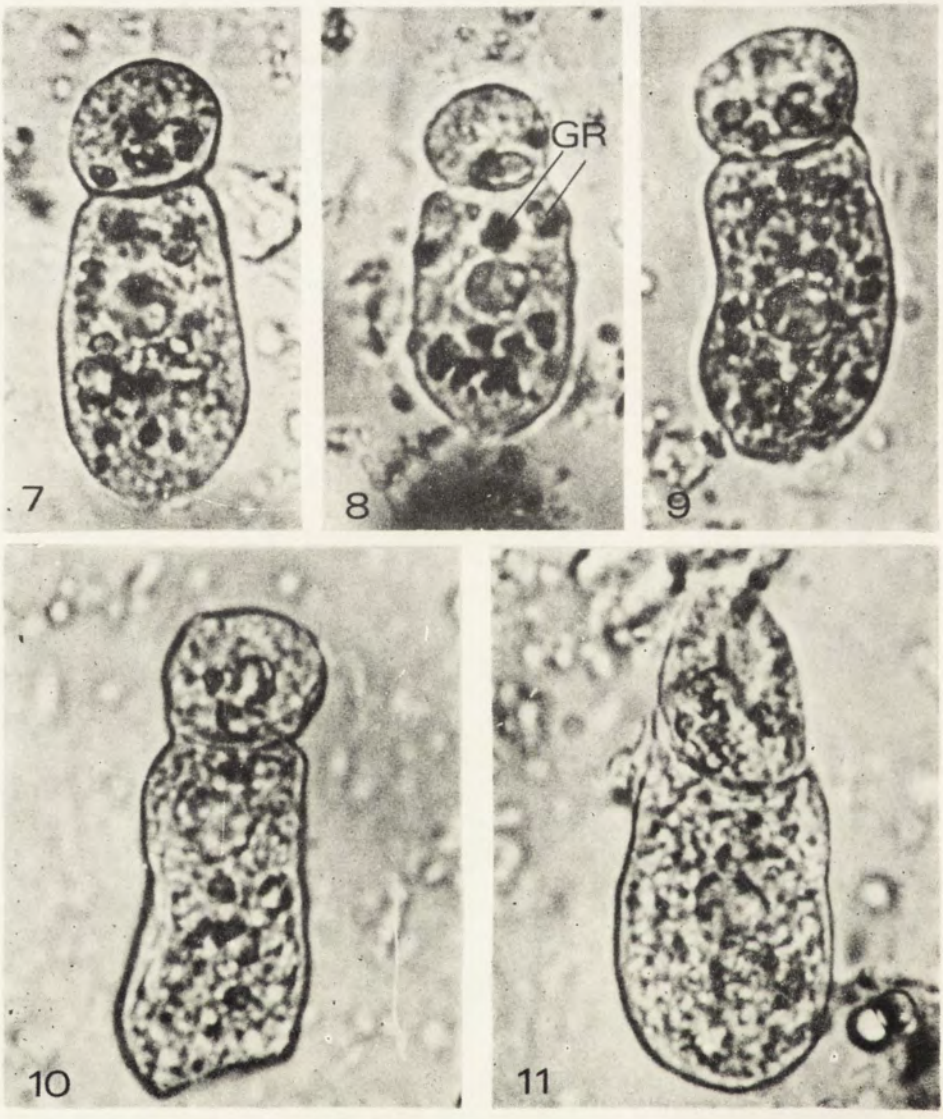

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Institute of Zoology, boul. Ruski, No. 1, Sofia, Bulgaria

Vassil GOLEMANSKY

Two New Isospora Species (*Coccidia: Eimeriidae*) Found in Wild Birds from Bulgaria

Synopsis: This report gives the description of two new coccidian species of the genus *Isospora*, found in wild birds from Bulgaria: *Isospora lusciniae* n. sp. (Host: *Luscinia megarhynchus* Brehm) and *Isospora sittae* n. sp. (Host: *Sitta europea* L.). A morphometric characteristics and data concerning the sporulation time of the oocysts of the described species as well as the location in the host and the habitats of the investigated birds are presented.

Isospora lusciniae n. sp. Pl. I 1, 2, 3

Description

The oocysts are round and colourless. The endocyst is darker in colour. The diameter of 44 measured oocysts ranges from 15.5 to 22.1 μm with an average of 18.8 μm.

The sporulation of the oocysts at a temperature of 24°C ± 1° is completed for 42–48 h. 1–2 and sometimes 4–5 polar granules spread among the sporocysts are observed after the sporulation.

The sporocysts are elongate-ellipsoidal and tapering at the front edge. At this edge a well expressed Stiedae body is observed. The size of the sporocysts ranges from 15.2–17.0×7.5–8.5 μm. The sporocyst residuum is dispersed among the sporosoites.

Type host: nightingale (*Luscinia megarhinchus* Brehm.).

Location: the oocysts were found in the small intestines.

Habitat: District of Blagoevgrad, Southwestern Bulgaria, 10.V.1976.

Discussion

Isospora lusciniae n. sp. is the only coccidian species found in the birds of the genus *Luscinia* till now. From the fact that the parasite was found in both of the investigated hosts with a medium intensity of invasion (1–5 oocysts per a square of the range of vision of a microscope with an eye-lens of 10 × and an object-lens of 16 ×) we may conclude

that this is not an accidental observation of oocysts from another host. *I. lusciniae* n. sp. differs from the widely spread in the warblers *I. lacazei* (Labbé) by the small size of the oocysts and the frequent observation in them of more polar granules, spread among the sporocysts.

Isospora sittae n. sp. Pl. I 4

Description

The oocysts are round, on rare occasions subspherical and colourless. Their diameter ranges from 23 to 30.4 μm with an average of 26.7 μm. The protoplasmal body is big and almost fills up the space of the oocyst.

The sporulation is completed for 48 h at a temperature of 25° ± 1°C. No residuum in the sporulated oocysts. A relatively big polar granule located among the sporocysts can be observed in most of the oocysts (Pl. I 4).

The sporocysts are elongate-ellipsoidal and tapering at one of the poles, where a well developed Stiedae body is to be found. A well developed hyaline substance can be observed at the front edge of the sporocysts, behind the Stiedae body. The size of the oocysts ranges from 11.8 to 13 μm in breadth and from 19.5 to 21.5 μm in length with an average of 12.4×20.5 μm. Among the sporosoites are observed several big granules — the dispersed residuum.

Type host: nuthatch (*Sitta europea* (L.)).

Location: in the small intestines.

Intensity of invasion: *I. sittae* n. sp. was observed in one of the investigated birds with a high intensity of invasion: 26 oocysts on the average in the range of vision of a microscope with 10× eye-lens and 16× objective lens.

Habitat: the mountain of Sredna Gora, 12.V.1976.

Discussion

The oocysts and the sporocysts of *I. sittae* n. sp. resemble the morphology of the second type oocysts of *I. lacazei* (Labbé) from the tits and other warblers, whose diameter ranges from 23 to 30 μm (Scholtyseck 1954, Schwalbach 1959, Pellerdy 1974 etc.). *I. sittae* differs from *I. lacazei* in the size of the sporocysts and in the presence in them of a well shaped hyaline substance, located behind the Stiedae body.

ACKNOWLEDGEMENTS

I express my gratitude to the ornithologists Dr. D. Nankinov from the Institute of Zoology in Sofia and Dr. Tz. Petrov from the Natural History Museum of Plovdiv for their kindness to present me materials from wild birds for a parasitological investigation.

RÉSUMÉ

Dans le présent travail sont décrites deux nouvelles espèces du genre *Isospora*, parasites des oiseaux en Bulgarie, a savoir: *I. lusciniae* n. sp. (H.: *Luscinia megarhinchus* Brehm.) et *I. sittae* n. sp. (H.: *Sitta europea* (L.).

REFERENCES

Pellerdy L. 1974: Coccidia and Coccidiosis. Budapest, éd. Kiado, 1–959.
Scholtyseck E. 1954: Untersuchungen über die bei einheimischen Vogelarten vorkommenden Coccidien der Gattung *Isospora*. Arch. Protistenk., 100, 91–112.
Schwalbach G. 1959: Untersuchungen und Beobachtungen an Coccidien der Gattungen *Eimeria*, *Isospora* und *Caryospora* bei Vögeln mit einer Beschreibung von sechzehn neuen Arten. Arch. Protistenk., 104, 3, 431–491.

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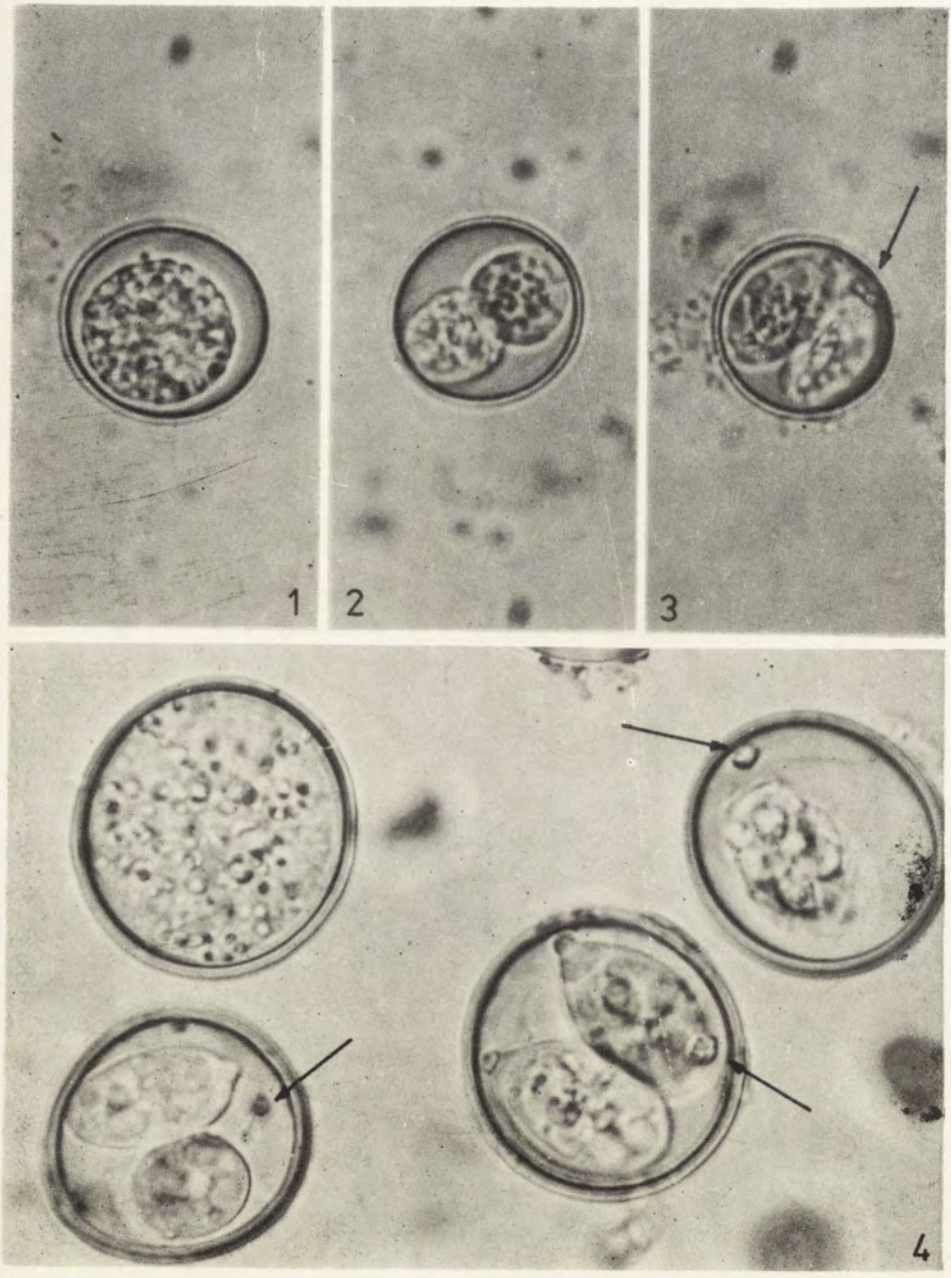
EXPLANATION OF PLATE I

1: *Isospora lusciniae* n. sp., unsporulated oocyst. × 1300
2: Id., state of two sporoblasts. × 1300
3: Id., sporulated oocyst. × 1300. The arrow points out the two polar granules
4: *Isospora sittae* n. sp., unsporulated and sporulated oocysts. × 1300. The arrowsws point out the polar granules



V. Golemansky auctor phot.

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Les Tintinnides (*Ciliata*: *Tintinnida*) des eaux marines et saumâtres du Québec. I. Formes hyalines [1]

Synopsis. Les Tintinnides de l'Estuaire et du Golfe du St-Laurent sont ici étudiés pour la première fois. L'analyse d'un ensemble de 144 échantillons de plancton récoltés dans la Baie des Chaleurs, le Fjord du Saguenay, les parties moyenne et basse de l'Estuaire du St-Laurent nous a montré 12 espèces appartenant à 7 genres. Des mesures de la longueur et de la largeur de la lorica ont été prises et sont présentées ici, accompagnées de caractéristiques concernant la distribution de ces espèces. L'on a pu noter que la Baie des Chaleurs montre une plus grande diversité que le Saguenay et l'Estuaire. Dans l'Estuaire moyen, nous n'avons observé aucun Tintinnides à lorica hyaline.

De toutes les études faunistiques sur les eaux marines ou saumâtres du Québec, aucune jusqu'ici n'a inclu les Tintinnides. Nous avons entrepris d'étudier d'une façon globale la distribution de ces organismes dans l'estuaire du St-Laurent, le Fjord du Saguenay et la Baie des Chaleurs. Nous nous en limitons dans ce premier temps seulement aux formes hyalines, dont la lorica est faite de sécretions organiques.

Matériel et méthodes

Nous avons utilisé pour cette étude 144 échantillons estivaux (mai à octobre) de plancton récoltés de 1971 à 1974 et répartis comme suit: 24 de l'estuaire moyen et 41 de l'estuaire maritime du St-Laurent, 27 de la Baie des Chaleurs et 52 du Fjord du Saguenay. Ces échantillons avaient alors été prélevés pour une étude qualitative du phytoplancton, et nous avons cru bon les reprendre pour les fins de la présente étude.

Les échantillons ont été recueillis à l'aide d'un filet standard (no. 25), avec un vide de maille de 64 µm. La sélectivité de cet engin fait en sorte que les

[1] Contribution au programme du G. I. R. O. Q. (Groupe interuniversitaire de recherches océanographiques du Quebec).

résultats présentés ici ne se veulent aucunement quantitatifs, mais servent plu plutôt de point de départ dans l'étude de ces Protozoaires ciliés.

Les deux caractères mesurés, la longueur de la lorica ainsi que le diamètre à son extrémité orale, sont inclus dans la description des espèces et sont exprimés de la façon suivante: longueur X diamètre. Mentionnons toutefois que les individus ont été comptés sur les lames de manière à obtenir un indice d'abondance relatif.

Résultats

Description des espèces

Nous avions d'abord identifié 18 espèces hyalines de Tintinnides, mais après consultation du travail de Burkovsky (1973), nous avons opté pour un regroupement de plusieurs espèces; ceci est particulièrement vrai pour le genre *Parafavella*, où nous avons regroupé 10 des espèces reconnues par Kofoid et Campbell (1929) en 3 espèces seulement. Cela a fait décroître le nombre total d'espèces hyalines de 18 à 12, dont nous fournissons ici une brève description ainsi qu'une illustration.

Ptychocylis drygalskii Brandt (Planche I, d)

Kofoid et Campbell (1929) p. 186 Fig. 350;
Marshall (1969) Pl. VI Fig. 21

Lorica de forme grossièrement conique dont le diamètre diminue considérablement en son tiers inférieur, pour montrer une forme arrondie à sa partie aborale.

Dimensions: 80–105×70–80 µm.

Localités: Saguenay, Estuaire maritime et Baie des Chaleurs.

Ptychocylis minor Jorgensen (Planche I, c)

Kofoid et Campbell (1929) p. 186 Fig. 354;
Marshall (1969) Pl. VI Fig. 23

Les 2/3 supérieurs de la lorica presque cylindriques, puis brusque diminution du diamètre pour former un pédicelle à la partie aborale.

Dimensions: 85–130×70–95 µm.

Localite: Estuaire maritime.

Ptychocylis acuta Brandt (Planche I, e)

Kofoid et Campbell (1929) p. 186 Fig. 353;
Marshall (1969) Pl. VI Fig. 17

Plus long que les deux précédents, sa partie inférieure est plus aiguë que chez *P. drygalskii* mais ne forme pas un pédicelle tel qu'observé chez *P. minor*.

Dimensions: 120–130×65–75 µm.

Localités: Saguenay, Estuaire maritime et Baie des Chaleurs.

Metacylis mereschkowskii Kofoid et Campbell (Planche III, p)

Kofoid et Campbell (1929) p. 198 Fig. 377;
Marshall (1969) sect. V p. 5

Lorica grossièrement conique, à peine plus longue que le diamètre oral: A cette extrémité, l'on peut distinguer des bandes disposées en anneaux sur le pourtour.

Dimensions: 45–60×30–35 µm.

Localités: Estuaire maritime et Baie des Chaleurs.

Acanthostomella gracilis Brandt (Planche I, a)

Kofoid et Campbell (1929) p. 192 Fig. 360;
Marshall (1969) Pl. VII Fig. 6

Lorica petite, grossièrement cylindrique et dont l'extrémité aborale se termine par une courte pointe. De plus l'extrémité orale est munie de denticules sur tout le pourtour.

Dimensions: 45–60×30–35 µm.

Localités: Estuaire maritime et Baie des Chaleurs.

Coxliella longa (Brandt) Laackman (Planche II, g)

Kofoid et Campbell (1929) p. 101 Fig. 196;
Marshall (1969) Pl. V Fig. 2

Lorica formée de bandes agencées en spirale et qui présente un renflement au niveau du tiers inférieur, avant de se fermer à l'extrémité aborale.

Dimensions: 140×60 µm.

Localite: Baie des Chaleurs.

Coxliella pseudoannulata (Jorgensen) Brandt (Planche II, h)

Kofoid et Campbell (1929) p. 98 Fig. 191;
Marshall (1969) Pl. V Fig. 12

Lorica formée de bandes annelées, agencées en spirale. De forme presque cylindrique, son diamètre diminue brusquement pour fermer l'extrémité aborale.

Dimensions: 105×50 µm.

Localite: Baie des Chaleurs.

Helicostomella subulata (Ehrenberg) Jorgensen (Planche I, f)

Kofoid et Campbell (1929) p. 106 Fig. 209;
Marshall (1969) Pl. V Fig. 25

Long cylindre se terminant à l'extrémité aborale par un long et mince pédicelle. De l'extrémité orale originent des bandes disposées en spirale sur le pourtour de la lorica et pouvant couvrir jusqu'aux 2/3 de la longueur totale de l'individu; ces spires sont munies de denticules.

Dimensions: 150–550×20–25 µm.

Localités: Saguenay, Estuaire maritime et Baie des Chaleurs.

Parafavella acuminata Ehrenberg

Marshall (1969) Pl. VIII Fig. 1

Lorica ponctuée de pores hexagonaux, comme c'est le cas pour les autres espèces du genre *Parafavella*, cylindrique sur les 2/3 de sa longueur, puis le diamètre diminue pour former l'extrémité aborale; à l'extrémité orale, le pourtour ne porte pas de denticules.

Dimensions: 70×50 µm.

Localités: Estuaire maritime et Baie des Chaleurs.

Parafavella denticulata (Ehrenberg) *sensu* Brukovsky comprenant
P. media (Brandt) Kofoid et Campbell

Kofoid et Campbell (1929) p. 160 Fig. 307
Marshall (1969) Pl. VIII Fig. 14

P. gigantea (Brandt) Kofoid et Campbell

Kofoid et Campbell (1929) p. 160 Fig. 301
Marshall (1969) Pl. VIII Fig. 10

P. cylindrica (Jorgensen) Kofoid et Campbell

Kofoid et Campbell (1929) p. 162 Fig. 312
Marshall (1969) Pl. VIII Fig. 5

P. edentata (Brandt) Kofoid et Campbell

Kofoid et Campbell (1929) p. 160 Fig. 296
Marshall (1969) Pl. VIII Fig. 8

P. denticulata (Ehrenberg) *sensu* Kofoid et Campbell

Kofoid et Campbell (1929) p. 160 Fig. 310
Marshall (1969) Pl. VIII Fig. 6

P. parumdentata (Brandt) Kofoid et Campbell

Kofoid et Campbell (1929) p. 168 Fig. 306
Marshall (1969) Pl. VIII Fig. 17

P. acuta (Jorgensen) Kofoid et Campbell

Koifoid et Campbell (1929) p. 158 Fig. 308
Marshall (1969) Pl. VIII Fig. 2

Pour les besoins du présent travail, nous avons tenté de reconnaître chacune des espèces regroupées par Burkovsky (1973) sous *P. denticulata*, pour lesquelles nous produisons les dimentions que nous avons relevées, ainsi qu'une illustration. Selon Burkovsky, un certain nombre d'espèces reconnues par Kofoid et Campbell (1929) entreraient dans la grande cyclomorphose annuelle de *P. denticulata*. Ce sont:

P. media (135–155×75–85 µm) (Planche II, i)
P. gigantea (220–575×60–80 µm) (Planche III, n)
P. cylindrica (220–350×60–70 µm) (Planche III, o)
P. edentata (95–330×40–70 µm) (Planche III, l)
P. denticulata (150–250×50–65 µm) (Planche II, k)

De plus, la grande similarité morphologique liant *P. parumdentata* (100–220×45–65 µm) Pl. II, j ainsi que *P. acuta* (125–265 C 65–70 µm) Pl. I, b au groupe mentionné ci-haut nous a incité à les y inclure.

Si nous regroupons les caractéristiques communes à toutes ces formes, nous obtenons une description très imprécise; en effet, la lorica peut présenter une très grande variabilité morphologique, comme on peut le constater en examinant les différentes illustrations. Cette lorica voit ses dimensions varier considérablement, peut être munie ou non d'un pédicelle et de denticules sur le pourtour oral.

Burkovsky (1973) a en effet observé, pour plusieurs caractères de la lorica, tous les intermédiaires d'un extrême à l'autre, d'où l'existence d'une seule espèce, *P. denticulata*. Ainsi, le nombre de denticules sur le pourtour oral, de grand qu'il est chez *P. denticulata*, diminue graduellement pour enfin devenir nul chez *P. edentata*. Il en est de même pour d'autres caractères de la lorica tels le diamètre oral et la longueur du pédicelle. Selon Burkovsky, les paramètres déterminants du milieu seraient la température de l'eau et l'abondance de la nourriture disponible.

Dimensions: 95–575×40–85 µm.

Localités: Saguenay, Estuaire moyen et Estuaire maritime

Parafavella elegans (Ostenfeld) Kofoid et Campbell

Kofoid et Campbell (1929) p. 160 Fig. 301
Marshall (1969) Pl. VIII Fig. 11

Lorica cylindrique sur 1/3 de sa longueur totale puis diminution lente du diamètre pour former un pédicelle plus ou moins long. Pourtour oral denticulé.

Dimensions: 125×50 µm.

Localités: Estuaire maritime et Baie des Chaleurs.

Favella composita Jorgensen (Planche III, m)

Kofoid et Campbell p. 148 Fig. 283

Lorica grossièrement conique, qui atteint sa largeur maximale au niveau du tiers inférieur, avant de se fermer à l'extrémité aborale. Pourtour oral non denticulé.

Dimensions: 120×50 µm.

Localité: Baie des Chaleurs.

Distribution

Comme nous l'avons mentionné plus haut, notre aire d'étude se subdivise en quatre régions: la Baie des Chaleurs, le Fjord du Saguenay, l'estuaire maritime et l'estuaire moyen du St-Laurent.

Tableau 1

Fréquence d'apparition des différentes espèces de Tintinnides à lorica hyaline dans chacune des régions étudiées

Region / Especes	Saguenay	Est. Moy.	Est. Mar.	Baie Chal.
Ptychocylis drygalskii	16	0	12	11
P. minor	0	0	28	0
P. acuta	21	0	4	12
Parafavella acuminata	0	0	5	3
P. denticulata (sensu lato)	2	0	4	18
P. denticulata (K. et C.)	0	0	0	14
P. gigantea	0	0	1	14
P. media	1	0	2	0
P. edentata	1	0	1	11
P. parumdentata	0	0	0	11
P. cylindrica	0	0	0	8
P. acuta	0	0	0	2
P. elegans	0	0	4	1
Coxliella longa	0	0	0	1
C. pseudoannulata	0	0	0	1
Metacylis merschkowskii	0	0	0	1
Acanthostomella gracilis	0	0	1	3
Helicostomella subulata	0	0	8	14
Favella composita	0	0	1	0
Total	39	0	67	65
Nombre de Prelevements	52	24	41	27

Le Tableau 1 représente la fréquence d'apparition de chaque espèce dans les quatre régions, c'est-à-dire le nombre de prélèvements dans lesquels on a retrouvé chacune de ces espèces. Ainsi, *Ptychocylis minor* a été observé dans 28 prélèvements de l'estuaire maritime, et ne l'a

été dans aucun échantillon provenant des autres régions à l'étude, alors que *P. drygalskii* et *P. acuta* se retrouvent dans le Saguenay, l'estuaire maritime et la Baie des Chaleurs.

Nous remarquons également que *A. gracilis* n'est présent que dans trois prélèvements issus de la Baie des Chaleurs; c'est cependant l'espèce dominante à la fin du mois de mai, d'après les estimés semi-quantitatifs que nous avons effectués. De même, *H. subulata,* bien que présente durant toute la saison estivale dans la Baie des Chaleurs, n'est dominante qu'en juillet, si l'on s'en tient aux formes retenues dans le filet.

De plus, l'ensemble des formes regroupées sous le nom de *Parafavella denticulata* se retrouve en grande majorité dans la Baie des Chaleurs, et sont très faiblement représentées ailleurs. Les trois formes les plus abondantes sont *P. gigantea, P. denticulata* et *P. parumdentata.* Seule *P. gigantea* montre un maximum d'abondance, et ce en juin, août et septembre. Les deux autres sont bien représentées mais ne dominent dans aucun des prélèvements.

Quant aux autres espèces, on ne les retrouve que dans la Baie des Chaleurs et dans l'estuaire maritime du St-Laurent.

L'étude du Tableau 1 dans son ensemble montre que la fréquence globale de présence ainsi que la diversité des formes hyalines de Tintinnides sont plus élevées dans la Baie des Chaleurs, où l'on admet qu'en moyenne la température et la salinité sont plus fortes que dans les autres régions étudiées (Lauzier 1957). La quantité d'organismes observés décroît dans l'estuaire maritime et le Saguenay, tandis que l'on enregistre aucune présence de Tintinnides à lorica hyaline dans l'estuaire moyen du St-Laurent; dans les deux premières régions, la température et la salinité sont plus faibles, et la turbidité plus élevée que dans la Baie des Chaleurs (Therriault 1973), tandis que la faible salinité caractéristique de l'estuaire moyen peut à elle seule expliquer l'absence de ces organismes.

SUMMARY

In this paper Tintinnids of the Estuary and Gulf of St. Lawrence are considered for the first time. The analysis of a collection of 144 plankton samples from the Baie des Chaleurs area, the Saguenay Fjord, the Upper and Lower St. Lawrence Estuary has shown the presence of 12 species belonging to 7 genera. Measurements of the length and width of lorica as well as distribution characteristics of these species are given. One can conclude the Baie des Chaleurs exhibits the highest diversity compared with the Saguenay and the Estuary. In the Upper Estuary, there is no Tintinnid with hyaline lorica.

BIBLIOGRAPHIE

Burkovsky I. V. 1973: Izmenčivost' infuzorii *Parafavella denticulata v Belom more*. Zool. Zh., 52, 1277–1285.

Burkovsky I. V. 1974: Raspredeleniye infuzorii *Parafavella denticulata* v Belom more. Zool. Zh., 53, 821–825.

Burkovsky I. V., Zamyshlyak E. A. et Poskryakova N. P. 1974: Revisiya fauny *Tintinnida* (*Ciliata*) Belogo Morya. Zool. Zh., 5, 1757–1766.

Jorgensen E. 1924: Mediterranean *Tintinnidae*. Rep. Dan. Oceanogr. Exped., 2, 1–110.

Kofoid C. A. et Campbell A. S. 1929: A Conspectus of Marine and Fresh-Water *Ciliata* belonging to the Suborder *Tintinnoinea* with descriptions of the new species principally from the Agassiz Expedition to the Eastern Tropical Pacific (1904–1905). University of California Press, Berkeley, California, 1–403.

Kofoid C. A. 1939: The *Ciliata*, The *Tintinnoinea*. Bull. Mus. Comp. Zool. Harvard Univ.

Lauzier L. 1957: Variations of Temperature and Salinity in Shallow Waters of the Southwestern Gulf of St-Lawrence. Bull. Fish. Res. Bd. Canada, 111, 251–268.

Marshall S. M. 1934: The *Silicoflagellata* and *Tintinnoinea*. Scient. Rep. Gt. Barrier Reef Exped. 4, 623–664.

Marshall S. M. 1969: *Protozoa*, Order *Tintinnida*. Cons. Int. Explor. Mer, 117–127.

Therriault J. C. 1973: Variations des propriétés physico-chimiques et biologiques d'une zone de mélange de l'estuaire du St-Laurent. Thèse de maîtrise, Université Laval, Faculté des Sciences, 154 pp.

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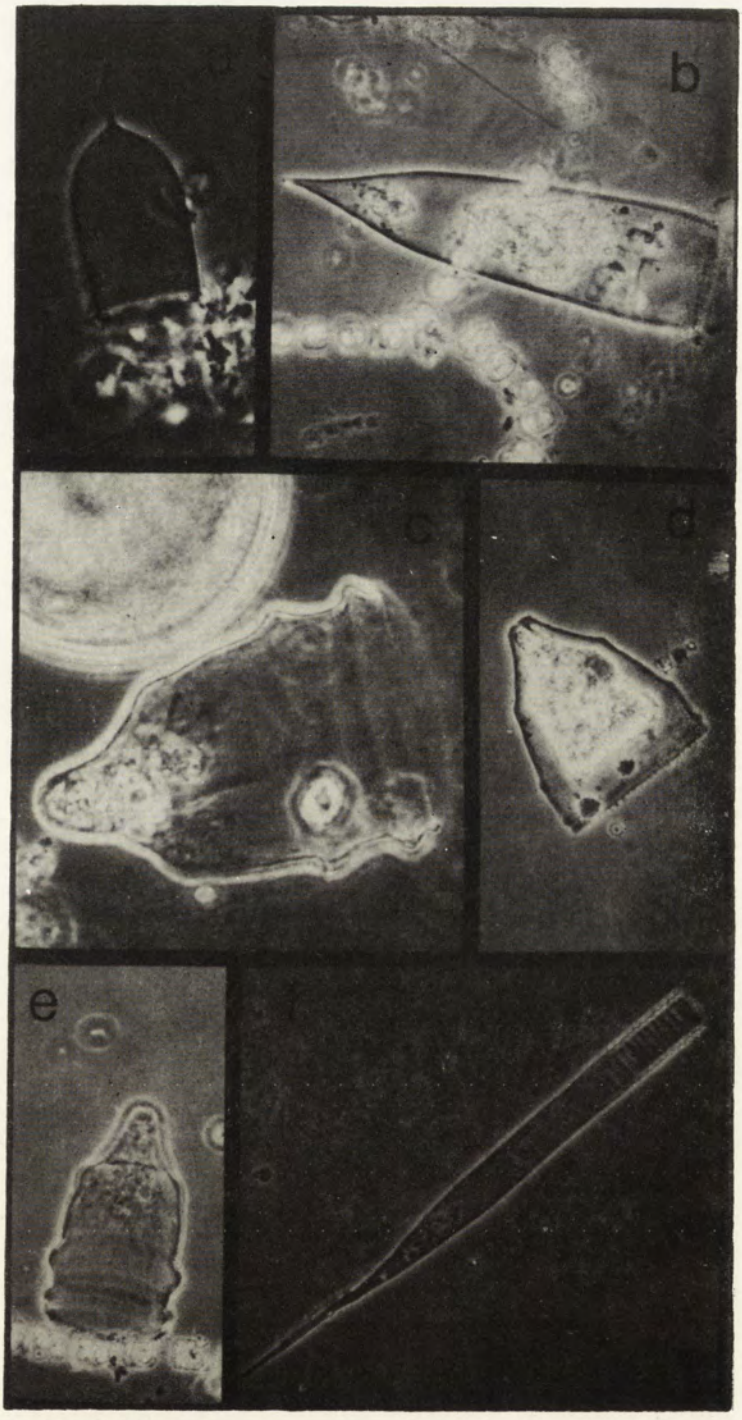
EXPLICATIONS DES PLANCHES I–III

a: *Acanthostomella gracilis* Brandt (× 400)
b: *Parafavella acuta* (Jorgensen) Kofoid et Cambbell (× 200)
c: *Ptychocylis minor* Jorgensen (× 400)
d: *Ptychocylis drygalskii* Brandt (× 200)
e: *Ptychocylis acuta* Brandt (× 200)
f: *Helicostomella subulata* (Ehrenberg) Jorgensen (× 200)
g: *Coxliella longa* (Brandt) Laackman (× 200)
h: *Coxliella pseudoannulata* (Jorgensen) Brandt (× 400)
i: *Parafavella media* (Brandt) K. et C. (× 400)
j: *Parafavella parumdentata* (Brandt) K. et C. (× 200)
k: *Parafavella denticulata* (Ehrenberg) sensu K. et C. (× 200)
l: *Parafavella edentata* (Brandt) K. et C. (× 200)
m: *Favella composita* Jorgensen (× 200)
n: *Parafavella gigantea* (Brandt) K. et C. (× 200)
o: *Parafavella cylindrica* (Jorgensen) K. et C. (× 200)
p: *Metacylis mereschkowskii* K. et C. (× 200)



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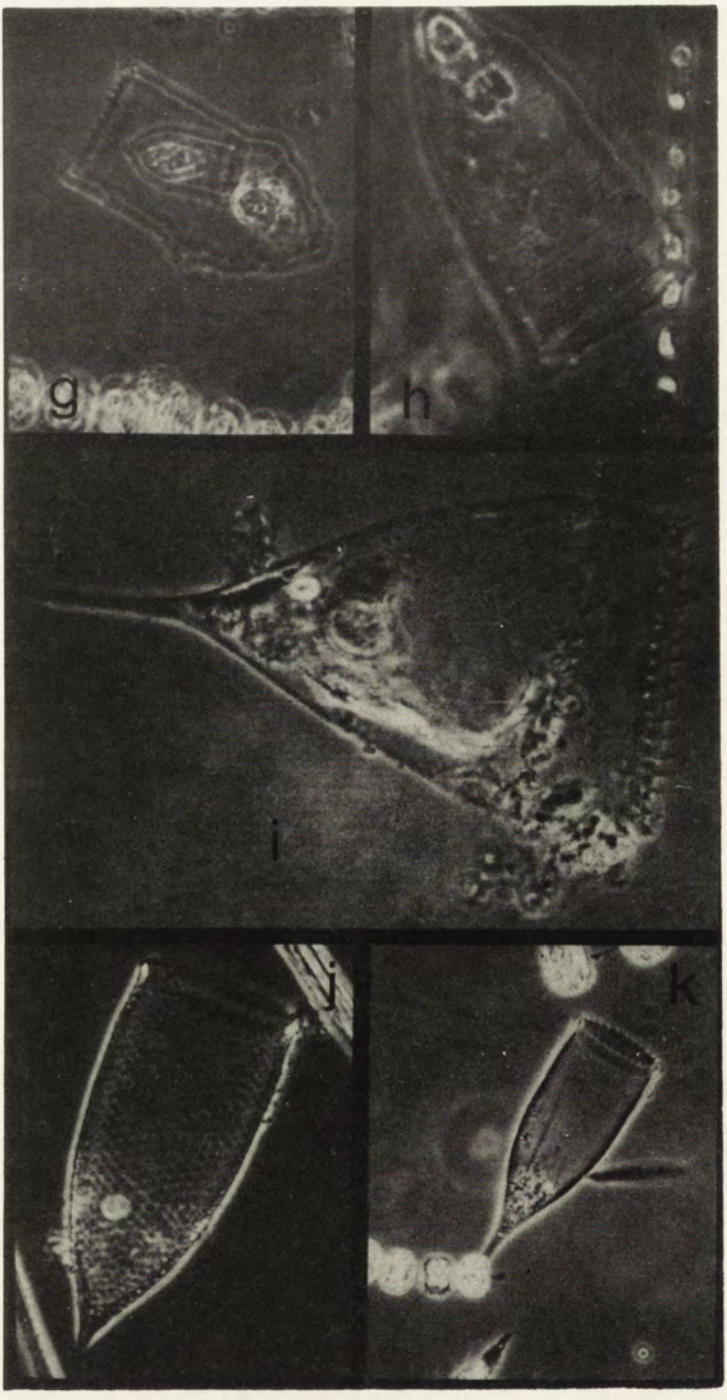

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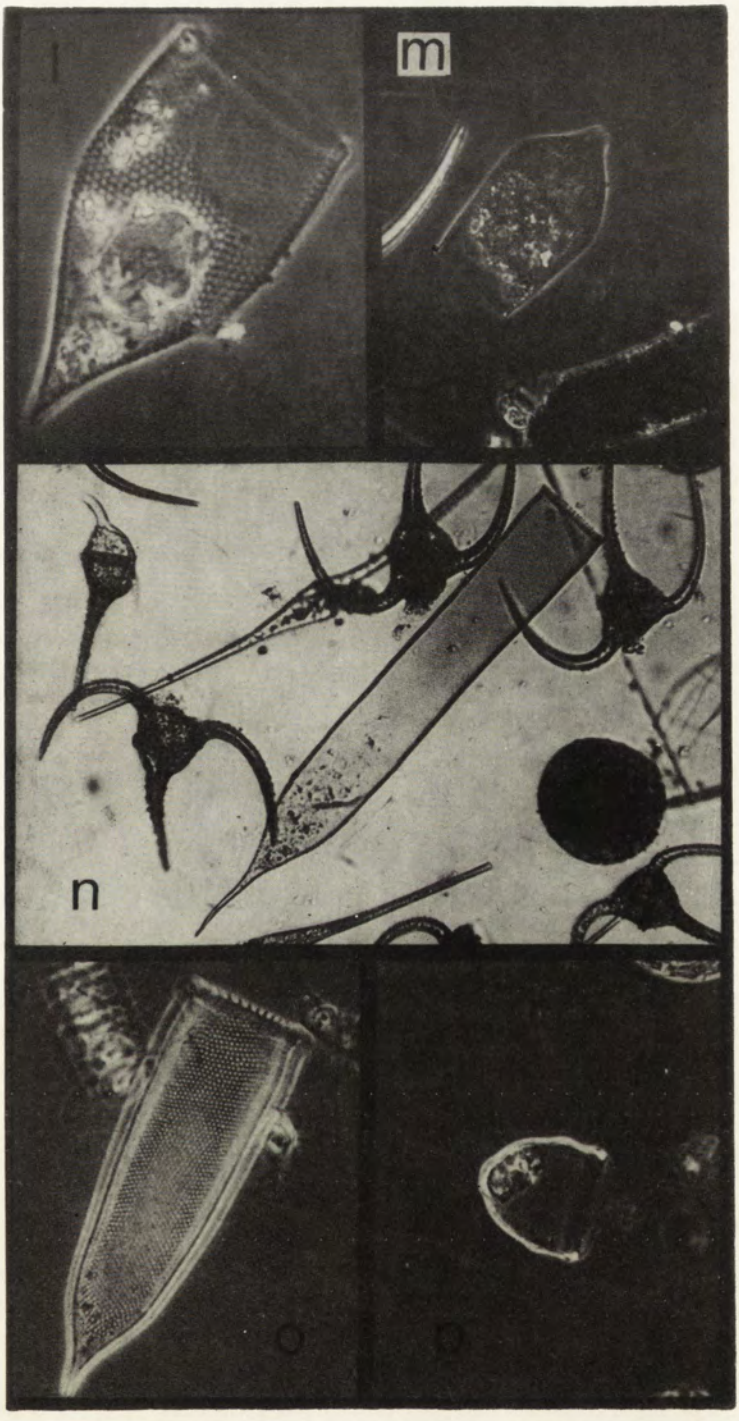

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Влияние денуклеации на фагоцитоз амеб

The Effect of Enucleation on Phagocytosis in Amoebae

Синопсис. При удалении ядра и при инактивации его актиномицином Д (1 мг/мл, 3-часовая экспозиция) у амеб наблюдается постепенная утрата способности захватывать пищевые организмы. Время полной утраты способности захватывать пищу различно при этих двух способах денуклеации. Поэтому одного прекращения синтеза и поступления в цитоплазму иРНК недостаточно для того, чтобы объяснить более быструю потерю пищевой реакции при механической денуклеации амеб. Высказывается предположение о значении „челночной" миграции некоторых ядерных белков и/или РНК между ядром и цитоплазмой для процесса фагоцитоза у амеб. Выдвинутое предположение не исключает возможной роли других факторов (прикрепление к субстрату, движение, состояние клеточной мембраны и др.) в этом процессе.

Роль ядра в той или иной функции клетки обычно оценивают, лишая клетку ее ядра (механическая денуклеация) или инактивируя его тем или иным способом (радиационная, химическая и т. п. денуклеация). Амебы особенно удобны для такого рода опытов.

Опыты с механической денуклеацией амеб дали ценную информацию о роли ядра в целом ряде жизненно-важных процессов в клетке, например, прикреплении к субстрату, движении, пиноцитозе, образовании сократительной вакуоли, выживаемости цитоплазмы и поддержании тонкой структуры цитоплазматических органелл (Chapman-Andresen 1962, Chapman-Andresen and Prescott 1956, Jeon 1968, Ord 1968, Flickinger 1968 a, Flickinger and Coss 1970).

Данные о роли ядра в фагоцитозе амеб довольно противоречивы. Согласно одним авторам (Браше 1960), денуклеированные половинки *A. proteus* не способны питаться живой добычей. Hirshfield (1959) в пищеварительных вакуолях безъядерных фрагментов амеб наблюдал захваченных и переваренных парамеций. У другого вида, *A. sphaeronucleus* (= *Thecamoeba sphaeronucleolus*), захват убитых дрожжей был довольно активным на протяжении

60 час. после удаления ядра (Comandon et de Fonbrune 1939 a). После реануклеации безъядерных фрагментов *A. sphaeronucleus* вслед за быстрым восстановлением движения наблюдался первый захват пищевых организмов амебами (Comandon et de Fonbrune 1939 b). Введение ядра в денуклеированную цитоплазму *A. proteus* приводило к временной реактивации, за которой следовал период неактивности, заканчивающийся либо гибелью амеб, либо постоянной реактивацией с питанием, ростом и делением (Ord 1968). На основании опытов с длительным выдерживанием в растворах актиномицина Д и пуромицина была высказана гипотеза о наличии особой „поведенческой" иРНК, обеспечивающей выбор и захват пищи у амеб (Орловская 1973).

Задача нашей работы — определение роли ядра в фагоцитозе амеб при двух способах денуклеации — механической и химической, вызываемой актиномицином Д. Изучали также интенсивность фагоцитоза на протяжении клеточного цикла, в особенности, во время митоза и в ранней интерфазе, когда ядро амеб претерпевает ряд морфологических и биохимических преобразований.

Материал и методика

Работа выполнена на амебах штамма В *Amoeba* proteus, культивируемых при 25°С. О фагоцитозе амеб судили по количеству захваченных тетрахимен при 15- или 30-минутном пребывании в среде с кормом (Кованько и Сопина 1977). В опыты брали амеб непосредственно после слива среды с остатками корма, а также через 24 и 48 час. после этого, т. е. соответственно через 24, 48 и 72 час. после последнего кормления.

Механическую денуклеацию осуществляли разрезанием амеб на две примерно равные по размеру половинки при помощи тонкой стеклянной нити. Безъядерные фрагменты очень быстро после операции откреплялись от дна микроаквариумов, округлялись, обездвиживались, и псевдоподии у них образовывались лишь спорадически. Фрагменты сортировали на безъядерные и содержащие ядро по их внешнему виду через 1 час после разрезания амеб.

Химическую денуклеацию амеб производили актиномицином Д (1 мг/мл), который в этой концентрации, как известно (Rao and Prescott 1970), полностью подавляет синтез РНК за 18 мин. Амеб выдерживали в растворе актиномицина Д в течение 0.5, 1.5 и 3.0 час. Интенсивность фагоцитоза амеб определяли после трехкратной отмывки от антибиотика на протяжении 6 час. в одних опытах и в течение 7 суток в других опытах при кормлении через день и ежедневной смене среды.

„Сферы деления" (клетки на стадии митоза) отбирали из массовой культуры после обильного кормления, через несколько минут после смены культуральной среды. „Сферы деления" по внешнему виду отличаются от интерфазных амеб: они более или менее сферические, откреплены от дна культурального сосуда, их поверхность покрыта короткими тупыми псевдоподиями. Исследовали фагоцитарную активность: (1) „сфер деления", (2) амеб, поделившихся во время пребывания в среде с кормом, и (3) амеб через различные промежутки времени после деления — 15, 30 и 60 мин., 12, 24 и 36 час. При изучении скорости фагоцитоза амеб на протяжении клеточного цикла на всех вышеупомянутых стадиях было изучено по 100 амеб.

Результаты

Безъядерные фрагменты

Среднее время сохранения жизнеспособности у безъядерных фрагментов (до их лизиса) — 5.9±0.1 суток. Результаты определения фагоцитарной активности безъядерных фрагментов на протяжении 6 суток после их получения представлены в Табл. 1. Видно, что скорость фагоцитоза безъядерных фрагментов ничтожна мала по сравнению с интенсивностью фагоцитоза контрольных половинок, содержащих ядро, и тем более целых амеб, не подвергавшихся разрезанию. Скорость фагоцитоза денуклеированных половинок падает до нуля на 4-е сутки после разрезания амеб. Однако и на протяжении 2–4 суток практически она также не отличается от нуля. Из данных,

Таблица 1
Table 1

Скорость фагоцитоза безъядерных фрагментов амеб в течение нескольких суток после денуклеации
The Phagocytosis Rate in Enucleated Fragments of Amoebae during Several Days after Enucleation

Тип амеб Type of amoebae	Время между разрезанием и тестированием Interval between cutting and testing	Среднее количество тетрахимен, захваченных за 30 мин. пребывания амеб в среде с кормом (в шт. на 1 амебу) Mean number of tetrahymenas taken up during 30 min stay of amoebae in food containing medium (per one amoeba)
Безъядерные фрагменты Enucleated fragments	0 суток (days)	0.13±0.05
	1	0.08±0.04
	2	0.05±0.05
	3	0.04±0.04
	4	0.00±0.00
	5	0.00±0.00
	6	0.00±0.00
Контроли: Control: (1) Фрагменты, содержащие ядро (1) Fragments with nucleus	5 час. 5 h	1.48±0.16
(2) Амебы с цитоплазматическим мостиком (2) Amoebae with cytoplasmic bridge	5 час. 5 h	2.26±0.28
(3) Целые амебы (3) Not injured amoebae	—	2.64±0.08

приведенных в таблице, следует, что амебы, которых подвергали неполному разрезанию, оставляя цитоплазматический мостик, соединяющий обе половинки, по своей фагоцитарной активности не отличаются от целых неоперированных амеб. Следовательно, сама операция по разрезанию не оказывает заметного влияния на скорость фагоцитоза амеб.

При длительном изучении безъядерных фрагментов амеб следует принимать во внимание влияние голодания на тот или иной тестируемый признак (Hirshfield 1959). Изменение скорости фагоцитоза амеб при длительном их голодании показано на Рис. 1. Видно, что наиболее активным фагоцитозом характеризуются амебы через 1–3 суток голодания. Начиная с 4-го дня наблюдается постепенное снижение интенсивности фагоцитоза, которая падает до нуля на 15–17-е сутки голодания. Среднее время выживания амеб (до их лизиса) при длительном голодании — 19.9 ± 0.3 суток. Но так как в опыт были взяты амебы через 48 час. после последнего кормления (через 24 час. после слива среды с остатками несъеденных тетрахимен), то среднее время выживания голодающих амеб следует увеличить, по крайней мере, еще на 1 сутки.

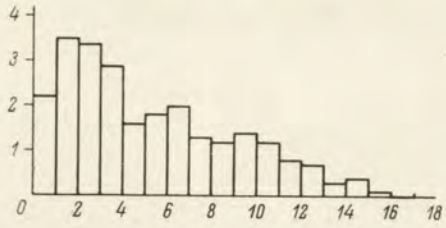

Рис. 1. Фагоцитоз амеб при длительном голодании. По оси абсцисс — время голодания (в сутках); по оси ординат — количество тетрахимен, захваченных за 30 мин. пребывания амеб в среде с кормом (в шт. на 1 амебу). 0-й день–24 часа после последнего кормления непосредственно после слива среды с кормом

Fig. 1. Phagocytosis of amoebae during long-term starvation. Abscissa, the time of starvation (days); ordinate, the mean number of tetrahymenas uptaken per amoeba during a 30 min exposure with food. Day 0–24 h after the last feeding, directly after removal of food-containing medium.

Таким образом, падение фагоцитарной активности до нуля у безъядерных фрагментов и целых голодающих амеб не совпадает во времени и разделено интервалом более чем в 2 недели. Следовательно, голодание не может быть причиной резкого снижения скорости фагоцитоза денуклеированных половинок амеб.

Результаты определения фагоцитарной активности безъядерных фрагментов на протяжении первых 6 час. после разрезания амеб представлены в Табл. 2. Наблюдается постепенный спад скорости фагоцитоза денуклеированных половинок с увеличением времени между разрезанием амеб и тестированием безъядерных фрагментов.

Аналогичные опыты были поставлены с определением фагоцитарной активности половинок, содержащих ядро, на протяжении первых 6 час. после их получения (Табл. 3). Из данных, приведенных в таблице, следует, что интенсивность фагоцитоза фрагментов, содержащих ядро, приблизительно

Таблица 2
Table 2

Скорость фагоцитоза безъядерных фрагментов амеб в течение первых часов после денуклеации
The Phagocytosis Rate of Enucleated Fragments of Amoebae during First Hours after Enucleation

Тип амеб Type of amoebae	Время между разрезанием и тестированием (в час.) Interval between cutting and testing in hours	Среднее количество тетрахимен, захваченных за 30 мин. пребывания амеб в среде с кормом (в шт. на I амебу) Mean number of tetrahymenas taken up during 30 min stay of amoebae in food containing medium (per one amoeba)
Безъядерные фрагменты Enucleated fragments	1	0.41 ± 0.04
	2	0.31 ± 0.07
	3	0.23 ± 0.05
	4	0.18 ± 0.05
	5	0.12 ± 0.03
	6	0.12 ± 0.02
Контроли: Control: (1) Фрагменты, содержащие ядро (1) Fragments with nucleus	5	188 ± 0.12
(2) Целые амебы (2) Not injured amoebae	—	3.64 ± 0.56

в 2 раза ниже скорости фагоцитоза целых амеб и остается неизменной в течение 6 час. после операции.

Если суммировать все данные, полученные на безъядерных и содержащих ядро фрагментах (все приблизительно через 5 час. после операции) и на целых амебах, то получим следующие цифры, характеризующие их скорости фагоцитоза: 0.12 ± 0.01, 1.93 ± 0.19 и 3.88 ± 0.43 захваченных тетрахимен на 1 фрагмент или 1 амебу, соответственно. Интенсивность фагоцитоза целых амеб в 2 раза превышает таковую фрагментов, содержащих ядро, которая, в свою очередь, приблизительно в 16 раз выше скорости фагоцитоза безъядерных фрагментов.

Таким образом, уже через 5 час. после удаления ядра половинки амеб почти полностью утрачивают способность захватывать пищу и характеризуются ничтожной фагоцитарной активностью по сравнению с фрагментами, содержащими ядро, и целыми амебами. Через сутки после денуклеации интенсивность фагоцитоза безъядерных фрагментов практически не отличается от нуля.

Таблица 3
Table 3

Скорость фагоцитоза фрагментов, содержащих ядро, в течение первых часов после разрезания амеб
The Phagocytosis Rate of Nucleate Fragments of Amoebae during First Hours after Their Cutting

Тип амеб Type of amoeba	Время между разрезанием и тестированием (в час.) Interval between cutting and testing (in hours)	Среднее количество тетрахимен, захваченных за 30 мин. пребывания амеб в среде с кормом (в шт. на 1 амебу) Mean number of tetrahymenas taken up during 30 min stay of amoebae in food containing medium (per one amoeba)
Фрагменты, содержащие ядро Nucleate fragments	1	2.15±0.31
	2	2.22±0.40
	3	2.19±0.43
	4	2.19±0.62
	5	2.29±0.52
	6	2.28±0.54
Контроли: Control: (1) Безъядерные фрагменты (1) Enucleated fragments	5	0.13±0.02
(2) Целые амебы (2) Not injured amoebae	—	5.02±0.65

Амебы после действия актиномицина Д

После 0.5-часового воздействия актиномицина Д судьба отмытых от антибиотика амеб была прослежена на протяжении недели, по сле 1.5 и 3-часовых экспозиций — в течение 27–28 суток (Табл. 4). Видно, что из трех испытанных сроков пребывания в актиномицине Д только 3-часовая экспозиция приводит к полному подавлению деления и, в конце концов, к гибели всех амеб, отмытых от антибиотика, т. е. по конечному результату эквивалентна механической денуклеации. Среднее время выживания амеб после 3-часового выдерживания в актиномицине Д — 9.4±0.3 суток.

Актиномицин Д во всех трех испытанных экспозициях (0.5, 1.5 и 3.0 час.) не оказывает заметного влияния на интенсивность фагоцитоза амеб в течение первых 6 час. после отмывки от антибиотика.

Результаты определения фагоцитарной активности амеб на протяжении 7 суток после отмывки от актиномицина Д представлены на Рис. 2. Оказалось, что после 0.5- и 1.5-часовых экспозиций в актиномицине Д скорость фагоци-

Таблица 4
Table 4

Судьба амеб после различных экспозиций в актиномицине Д (1 мг/мл)
The Fate of Amoebae after Different Exposures to Actinomycin D (1 mg/ml)

Экспозиция в актиномицине Д (в час.) Time of exposure to actinomycin D (in hours)	Время после отмывки от антибиотика Time after washing from antibiotic (in days)	% погибших амеб % of dead amoebae	% поделившихся амеб % of divided amoebae	% не поделившихся амеб % of not divided amoebae
0.5	7	10	74	16
1.5	7	4	48	48
	8–28	26+2*	22–2*	0
	За весь период for the whole period	32	68	0
3.0	7	16	0	84
	8–27	82	2	0
	За весь период for the whole period	98	2	0
Контроль (7 суток) Control (7 days)	—	0	87.5	2.5

* Доля погибших амеб из числа поделившихся за первую неделю.
* Part of amoebae which died after division during the first week.

тоза амеб постепенно снижается, на 3–4-е сутки характеризуется минимальной величиной, а затем постепенно повышается и на 5–7-е сутки не отличается от интенсивности фагоцитоза контрольных, не подвергавшихся воздействию антибиотика амеб. После 3-часового выдерживания в актиномицине Д скорость фагоцитоза амеб, постепенно снижаясь, падает практически до нуля на 4-е сутки после отмывки от антибиотика. Следует отметить, что одновременно с утратой способности захватывать пищу у этих амеб наблюдается и открепление от дна микроаквариумов.

Таким образом, из трех испытанных сроков пребывания в актиномицине Д только 3-часовая экспозиция, полностью блокирующая деление у отмытых от антибиотика амеб, лишает их способности захватывать пищевые организмы. После меньших экспозиций в актиномицине (0.5 и 1.5 час.) вслед за восстановлением деления (на 3–4-е сутки) наблюдается повышение интенсивности фагоцитоза отмытых от антибиотика амеб (на 4–5-е сутки).

Амебы во время деления и после него

У „сфер деления", не поделившихся к концу 30-минутного пребывания в среде с кормом, была отмечена такая же низкая фагоцитарная активность,

как и у безъядерных фрагментов — 0.22 ± 0.10 захваченных тетрахимен на 1 „сферу деления". Из 100 „сфер деления", взятых в опыт, к концу 30-минутного пребывания в среде с кормом поделились 73. Поделившиеся в среде с кормом амебы также характеризуются низкой фагоцитарной активностью — 0.10 ± 0.03 захваченных тетрахимен на 1 дочернюю амебу. Если принимать во внимание только „сферы" поделившиеся в течение 30 мин., то, в среднем, они делятся через 13.8 ± 1.0 мин. после переноса из массовой культуры в среду с кормом. Следовательно, большая часть только что поделившихся амеб находится в корме приблизительно 15 мин. Сравнение скоростей фагоцитоза этих амеб и контрольных амеб, взятых из массовой культуры непосредственно после слива среды с кормом, при 15-минутном пребывании в среде с кормом (0.96 ± 0.22) показывает, что делящиеся в присутствии корма амебы, так же как и „сферы деления", почти не питаются.

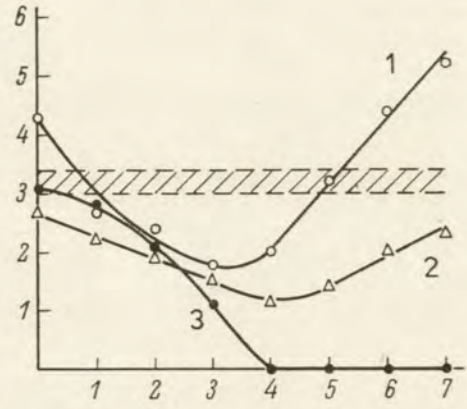

Рис. 2. Зависимость скорости фагоцитоза амеб, отмытых от актиномицина Д (1 мг/мл), от времени пребывания в антибиотике. По оси абсцисс — время после отмывки от актиномицина Д (в сутках); по оси ординат — количество тетрахимен, захваченных за 30 мин. пребывания амеб в среде с кормом (в шт. на 1 амебу). Заштрихованная зона — фагоцитоз контрольных, не подвергавшихся воздействию актиномицина Д амеб. Експозиции в актиномицине Д: *1* — 0.5 час., *2* — 1.5 час., *3* — 3.0 час

Fig. 2. The phagocytosis rate of amoebae washed from actinomycin D related to the time of actinomycin D exposure. Abscissa, the time after washing from actinomycin D (days); ordinate, the mean number of tetrahymenas uptaken per amoeba during a 30 min exposure with food. Shaded area means phagocytosis of control amoebae not exposed to actinomycin D. Actinomycin D exposures (hours): *1* — 0.5, *2* — 1.5, *3* — 3.0.

Продолжительность клеточного цикла у амеб штамма В при 25°С при кормлении через день и ежедневной смене среды — приблизительно 43 час. Результаты испытания скорости фагоцитоза амеб на протяжении клеточного цикла представлены в Табл. 5. У амеб через 15 и 30 мин. после деления фагоцитарная активность выше, чем у „сфер деления", но ниже, чем через 60 мин. после деления. Скорость фагоцитоза последних не отличается от таковой амеб через 12 час. после деления и контрольных амеб непосредственно после слива среды с остатками корма. Через 24 час. после деления скорость фагоцитоза амеб выше, чем через 12 час. после деления и не отличается от скорости захвата пищи контрольными амебами через 24 час. после слива среды с кормом. У амеб через 24 и 36 час. после деления скорости фагоцитоза достоверно не различаются между собой.

Следовательно, у „сфер деления" и у постмитотических амеб приблизительно через 15 мин. после цитокинеза наблюдается ничтожная фагоцитар-

Таблица 5
Table 5

Скорость фагоцитоза амеб через различное время после деления
The Phagocytosis Rate of Amoebae Examined at Different Time Intervals after Division

Время после деления Time after division	Среднее количество тетрахимен, захваченных за 30 мин. пребывания амеб в среде с кормом (в шт. на 1 амебу) Mean number of tetrahymenas taken up during 30 min stay of amoebae in food containing medium (per one amoeba)	t_d
15 мин. min	0.5 ± 0.1	1.43
30 мин. min	0.7 ± 0.1	
60 мин. min	1.7 ± 0.1	7.14
12 час. hours	1.7 ± 0.1	5.91
24 час. hours	3.0 ± 0.2	1.79
36 час. hours	3.5 ± 0.2	

ная активность, которая постепенно возрастает по мере увеличения времени между цитокинезом и началом тестирования и восстанавливается до уровня соответствующего контроля между 30 и 60 мин. после деления (между 60 и 90 мин. включая время пребывания в среде с кормом).

При испытании скорости фагоцитоза амеб на протяжении клеточного цикла контролями служили амебы, взятые из массовой культуры непосредственно после слива среды с кормом, а также через 24 и 48 час. после этого, т. е., соответственно, через 24, 48 и 72 час. после кормления. При 30-минутном пребывании в среде с кормом у амеб через 24 час. после кормления скорость фагоцитоза (1.9 ± 0.2) ниже, чем через 48 (3.1 ± 0.2) и 72 час. (3.5 ± 0.3) после кормления. Последние две цифры достоверно не различаются между собой.

Следовательно, непосредственно после слива среды с остатками корма фагоцитарная активность „сытых" амеб ниже таковой амеб, голодавших в течение 1–2 суток.

Обсуждение

Известно, что актиномицин Д блокирует синтез иРНК путем взаимодействия с ДНК по типу интеркаляции и создания препятствий на пути перемещения РНК-полимеразы вдоль матрицы ДНК (Ашмарин и Ключарев 1975). У *A. proteus* актиномицин Д (1 мг/мл) полностью подавляет синтез РНК за 18 мин. (Rao and Prescott 1970). Однако из трех испытанных экспозиций в актиномицине Д только 3-часовое выдерживание в антибиотике полностью и необратимо подавляло у амеб способность захватывать пищу. Можно думать, что при 0.5 и 1.5-часовых экспозициях связывание актиномицина Д с ядерной ДНК является обратимым, тогда как при 3-часовой экспозиции —

необратимым. Постепенное снижение скорости фагоцитоза до нуля после 3-часового выдерживания в актиномицине Д позволяет предполагать, что в условиях блока транскрипции фагоцитоз у амеб сохраняется до полного разрушения всех матриц предсинтезированных иРНК и синтезированных на них белков, имеющих отношение к этому процессу. Не исключено поэтому, что одним из факторов, обеспечивающих нормальный захват пищи амебами, является иРНК, синтезируемая в ядре и поступающая из ядра в цитоплазму. Аналогичный вывод был сделан ранее Орловской (1973) при изучении пищевой реакции у амеб после длительного их выдерживания в растворах актиномицина Д различной концентрации.

По нашим данным, среднее время выживания амеб, отмытых от актиномицина Д после 3-часовой экспозиции, превышает жизнеспособность безъядерных фрагментов, но значительно ниже времени выживания целых голодающих амеб. Время полного исчезновения фагоцитарной активности у амеб также различно при механическом удалении ядра и при инактивации его актиномицином Д. Аналогичные результаты были получены при сравнении сроков изменения и исчезновения цитоплазматических органелл (комплексов Гольджи и эндоплазматической сети) при обоих способах денуклеации амеб (Flickinger 1968 a, b). Так как скорости, с которыми сказываются на фагоцитозе механическое удаление ядра и инактивация его актиномицином Д, резко различаются, то трудно сводить участие ядра в процессах захвата пищи амебами только к синтезу и поставке в цитоплазму иРНК.

Несколько лет назад у *A. proteus* была открыта постоянная миграция некоторых ядерных белков и РНК между ядром и цитоплазмой (см. обзор: Goldstein 1973). Были обнаружены два класса мигрирующих ядерных белков: быстро мигрирующие белки и белки с медленной скоростью обновления (Goldstein and Prescott 1967). Для быстро мигрирующих ядерных белков характерна миграция между ядром и цитоплазмой в обоих направлениях. Эта миграция была названа „челночной" (англ. shuttling). Позднее было показано, что некоторые ядерные РНК также мигрируют не только из ядра в цитоплазму, но и в обратном направлении (Goldstein et al. 1969).

При механической денуклеации ядро удаляется из клетки. При химической его инактивации оно остается в клетке. При этом сохраняются некоторые стороны его взаимодействия с цитоплазмой. Так, движение РНК из ядра в цитоплазму в значительной степени, но не полностью подавляется актиномицином Д (имеется в виду РНК, снитезированная до начала действия актиномицина Д), в то время как миграция РНК из цитоплазмы в ядро подавляется лишь незначительно (Goldstein et al. 1969). Правда, при этом некоторые из предсинтезированных РНК вовсе перестают мигрировать из цитоплазмы в ядро (Goldstein and Trescott 1970).

Поэтому можно предположить, что нормальная миграция макромолекул между ядром и цитоплазмой существенна для многих функций клетки,

в том числе для процессов фагоцитоза. В пользу этого предположения говорит снижение фагоцитарной активности амеб в те периоды клеточного цикла, когда нарушается нормальная (для интерфазы) миграция макромолекул между ядром и цитоплазмой, а именно в митозе и в первые часы после митоза. Синтез ядерной РНК у *A. proteus* отсутствует с поздней профазы до ранней телофазы (Rao and Prescott 1970). Большая часть ядерных белков и РНК выходит из ядра делящейся клетки в поздней профазе (Prescott and Goldstein 1968, Rao and Prescott 1970, Yudin and Neyfakh 1973). Внутренний слой ядерной оболочки, исчезающий в профазе, появляется после телофазы, и значительная часть его наращивается только спустя 30 мин. после цитокинеза (Roth et al. 1960). Ядерные РНК возвращаются из цитоплазмы в ядра дочерних клеток очень быстро — в пределах 15 мин. после деления, тогда как для возвращения ядерных белков, вышедших в цитоплазму во время митоза, требуется 3 часа (Prescott and Goldstein 1968, Rao and Prescott 1970).

Сопоставление вышеизложенных данных со сроками восстановления нормальной фагоцитарной активности амеб после деления показывает, что это восстановление коррелирует с: (1) реконструкцией ядерной оболочки, (2) возвращением в ядро значительной части РНК и белков, вышедших в цитоплазму во время митоза, и (3) возобновлением нормальной миграции ядерных РНК и белков между ядром и цитоплазмой.

Предположение о значении „челночной" миграции макромолекул для нормального захвата пищи амебами не исключает возможной роли других факторов в этом процессе (прикрепление к субстрату, движение, состояние клеточной мембраны и др.).

Удаление ядра из *A. proteus* приводит, как известно, к быстрому открепнию амеб и утрате ими нормальной подвижности, а последующая ренуклеация сопровождается в равной степени быстрым восстановлением способности прикрепляться и двигаться (Comandon et de Fonbrune 1939 a, b, Jeon 1968). Быстрота, с которой амебы утрачивают и приобретают свою подвижность при механической денуклеации и последующей ренуклеации, заставила некоторых исследователей (Prescott 1960, Jeon 1968) отказаться от мысли, что какой-либо транспорт макромолекул из ядра в цитоплазму принимает участие в контроле амебоидного движения. Однако совсем недавно было высказано предположение (Goldstein 1973), что нормальное (для интерфазы) амебоидное движение зависит от непрерывного поступления из ядра в цитоплазму ядерных белков. При этом быстрый эффект удаления ядра или ренуклеации может объясняться высокой скоростью обновления этих белков и их перемещения между ядром и цитоплазмой (см.: Юдин 1976).

У безъядерных фрагментов, у „сфер деления" и у амеб на 4-е сутки после отмывки от актиномицина Д (3-часовая экспозиция) потеря способности захватывать пищу тесно коррелирует с утратой способности прикрепляться и ползать. Поэтому мы не можем дать однозначный ответ, является ли утрата

процессов фагоцитоза у амеб при обоих способах денуклеации прямым следствием самой денуклеации или она обусловлена потерей способности прикрепляться к субстрату и двигаться. В связи с этим существенно, что у амеб утрата пищевой реакции не всегда сопровождается потерей способности прикрепляться и двигаться. Так, после 24–36-часового выдерживания в растворах актиномицина Д амебы утрачивают способность захватывать пищевые „модели", но остаются прикрепленными к субстрату и сохраняют нормальную полиподиальную форму (Орловская 1973). В наших опытах только что поделившиеся амебы плотно прикреплены к субстрату, полиподиальны и активно движутся, но в течение короткого времени после деления характеризуются пониженной фагоцитарной активностью. Следовательно, хотя прикрепление и движение и являются необходимыми условиями фагоцитоза у *A. proteus*, тем не менее одного прикрепления и движения, по-видимому, недостаточно для осуществления амебами нормального захвата пищи.

Известно, что делящиеся клетки амеб не обнаруживают реакцию пиноцитоза (Prescott 1959). Непосредственно после деления пиноцитарная активность амеб очень низка; она постепенно возрастает до некоторого постоянного уровня в течение 1–2 час. после деления (Sanders and Bell 1970). После обильного кормления у амеб наблюдается не только пониженная фагоцитарная активность (Орловская 1973; наши собственные данные), но и слабая реакция на индукторы пиноцитоза (Chapman-Andresen 1963). По мнению Серавина (1970), для протекания процессов фагоцитоза и пиноцитоза требуется определенный резерв клеточной поверхностной мембраны, по исчерпании которого оба процесса затормаживаются. Одновременное снижение скоростей фагоцитоза и пиноцитоза у амеб непосредственно после деления, так же как и после обильного кормления, можно объяснить истощением резервов поверхностной мембраны в процессе деления или в процессе интенсивного фагоцитоза, в результате чего мембрана на некоторое время оказывается мало пригодной для процессов питания.

В связи с этим следует упомянуть о снижении скорости фагоцитоза содержащих ядро фрагментов в 2 раза по сравнению с таковой целых амеб. Среднее число пиноцитарных каналов в половинках, содержащих ядро, также приблизительно в 2 раза ниже величины, найденной для целых амеб (Chapman-Andresen 1962). Можно предполагать, что уменьшение количества поверхностной мембраны приблизительно в 2 раза у фрагментов, содержащих ядро, приводит к соответствующему пропорциональному снижению скоростей фагоцитоза и пиноцитоза. Однако высказанное предположение не исключает и другое возможное объяснение полученных результатов: снижение фагоцитарной активности фрагментов, содержащих ядро, может быть и следствием уменьшения количества находящихся в цитоплазме мигрирующих РНК и белков в результате ампутации половины цитоплазмы.

SUMMARY

A gradual loss of the capacity of uptaking food organisms (*Tetrahymena pyriformis*) is observed in amoebae after both a removal or actinomycin D — induced inactivation (1 mg/ml, 3 h exposure) of the nucleus. With these two ways of enucleation, the complete loss of the capacity of uptaking food was achieved at different time. This may suggest that the very cessation of mRNA synthesis and supply into cytoplasm is not sufficient to explain a quicker loss of the phagocytosis activity after the former way of enucleation. It is proposed that the shuttling of some nuclear proteins and/or RNA between the nucleus and cytoplasm is important, directly or indirectly, for phagocytosis of amoebae. This hypothesis does not exclude a possible role of other factors in this process, for example, attachment to the substratum, movement, condition of the cell membrane and others.

ЛИТЕРАТУРА

Ашмарин И. П. и Ключарев Л. А. 1975: Ингибиторы синтеза белка. Изд-во Медицина, Ленинград, 16-25.

Браше Ж. 1960: Биохимическая цитология. Изд-во Ин. лит., Москва, 313-374.

Chapman-Andresen C. 1962: Studies on pinocytosis in amoebae. C. r. Trav. Lab. Carlsberg, 33, 73–264.

Chapman-Andresen C. 1963: Pinocytosis in *Amoeba proteus*. Some observations on the utilisation of membrane during pinocytosis. In: Progress in Protozoology, Proc. First int. Congr. Protozool., Prague 1961, Publ. House Czechosl. Acad. Sci., Prague, 267–270.

Chapman-Andresen C. and Prescott D. M. 1956: Studies on pinocytosis in the amoebae *Chaos chaos* and *Amoeba proteus*. C. r. Trav. Lab. Carlsberg, sér. chim., 30, 57–78.

Comandon J. et de Fonbrune P. 1939 a: Ablation du noyau chez une Amibe. Réactions cinétiques a la piqûre de l'Amibe normale ou dénuclée. C. r. seanc. Soc. Biol., 130, 740–744.

Comandon J. et de Fonbrune P. 1939 b: Greffe nucléaire totale, simple ou multiple, chez une Amibe. C. r. seanc. Soc. Biol., 130, 744–748.

Flickinger C. J. 1968 a: The effects of enucleation on the cytoplasmic membranes of *Amoeba proteus*. J. Cell Biol., 37, 300–315.

Flickinger C. J. 1968 b: Cytoplasmic alterations in amoebae treated with actinomycin D. A comparison with the effects of surgical enucleation. Expl. Cell Res., 53, 241–251.

Flickinger C. J. and Coss R. A. 1970: The role of the nucleus in the formation and maintenance of the contractile vacuole in *Amoeba proteus*. Expl. Cell Res., 62, 326–330.

Goldstein L. 1973: Nucleocytoplasmic interactions in amoebae. In: The Biology of *Amoeba*. Academic Press, N. Y. and London, 479–504.

Goldstein L. and Prescott D. M. 1967: Proteins in nucleocytoplasmic interactions. I. The fundamental characteristics of the rapidly migrating proteins and the slow turnover proteins of *Amoeba proteus* nucleus. J. Cell Biol., 33, 637–644.

Goldstein L. and Trescott O. H. 1970: Characterization of RNAs that do and do not migrate between cytoplasm and nucleus. Proc. natn. Acad. Sci. USA, 67, 1367–1374.

Goldstein L., Rao M. V. N. and Prescott D. M. 1969: The migration of RNA from cytoplasm to nucleus in *Amoeba proteus*. Ann. Embryol. Morphog., Suppl. 1, 189–197.

Hirshfield H. I. 1959: Nuclear control of cytoplasmic activities. Ann. N. Y. Acad. Sci., 78, 647–654.

Jeon K. W. 1968: Nuclear control of cell movement in amoebae: nuclear transplantation study. Expl. Cell Res., 50, 467–471.
Кованько Е. Г. и Сопина В. А. 1977: Кинетика фагоцитоза амеб. Цитология (в печати).
Ord M. J. 1968: The viability of the anucleate cytoplasm of *Amoeba proteus*. J. Cell Sci., 3, 81–88.
Орловская Э. Э. 1973: О роли ядра в пищевой реакции у *Amoeba proteus*. Вестник Лен. Ун-та, 3, 20—27.
Prescott D. M. 1959: Microtechniques in amoebae studies. Ann. N. Y. Acad. Sci., 78, 655–661.
Prescott D. M. 1960: Nuclear function and nuclear-cytoplasmic interactions. In: Annual Review of Physiology, 22. Annu. Rev. Inc., Palo Alto, California, 17–44.
Prescott D. and Goldstein L. 1968: Proteins in nucleocytoplasmic interactions. III. Redistributions of nuclear proteins during and following mitosis in *Amoeba proteus*. J. Cell Biol., 39, 404–414.
Rao M. V. N. and Prescott D. M. 1970: Inclusion of predivision labeled nuclear RNA in post division nuclei in *Amoeba proteus*. Expl. Cell Res., 62, 286–292.
Roth L. E., Obetz S. W. and Daniels E. W. 1960: Electron microscopic studies of mitosis in amoebae. I. *Amoeba proteus*. J. Biophys. Biochem. Cytol., 8, 207–220.
Sanders E. J. and Bell L. G. E. 1970: Inhibition of pinocytosis in *Amoeba proteus* by puromycin. Expl. Cell Res., 63, 379–384.
Серавин Л. Н. 1970: Фагоцитоз и пиноцитоз. В кн.: Проблемы современной биологии. Изд-во Лен. Ун-та, Ленинград, 89—105.
Юдин А. Л. 1977: Движение макромолекул между ядром и цитоплазмой по данным опытов с амебами. В кн.: Проблемы экспериментальной биологии. Изд-во „Наука", Москва (в печати).
Yudin A. L. and Neyfakh A. A. 1973: Migration of newly-synthesized RNA during mitosis. IV. Content of labelled RNA in nuclei before and after mitosis in *Amoeba proteus*. Expl. Cell Res., 82, 210–214.

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Effect of Temperature on the Viability of Spores of *Thelohania orchestii* and *Pleistophora ganapatii*

Synopsis. The optimum temperature for the viability of the spores of *Thelohania orchestii* and *Pleistophora ganapatii* was found to be 25°C. The spores lost their viability rapidly at higher temperatures and death was instantaneous at 42°C in *Thelohania orchestii* and at 40°C in *Pleistophora ganapatii*. At low temperatures (between 12°C and 0°C) they lost their viability slowly and the spores were completely non viable in two days in the case of *Thelohania orchestii* and in 21 days in the case of *Pleistophora ganapatii* when they were exposed to 0°C.

Allen (1954), Allen and Brunson (1947), Finney et al. (1947), Karmo and Morganthaler (1939), Steinhaus and Hughes (1949), and Weiser (1961) studied the effect of both high and low temperatures on the spores of different microsporidians. Pojarkov (1940), Lotmar (1944), Ovanesjan and Lobzandze (1956, 1958) and Astaurov (1956) studied the effect of temperature with a view to find out a thermostatic control of the diseases caused by the microsporidians. In the present investigation an attempt has been made to study the effect of both high and low temperatures on the spores of two microsporidian parasites, *Thelohania orchestii* Kalavati, 1976 and *Pleistophora ganapatii* Kalavati, 1976, the former from the body muscles of a terrestrial amphipod, *Orchestia platensis* Kr. and the latter from the gut of a termite *Odontotermes horni* (Wasm.).

Material and Methods

The spores of *Thelohania orchestii* were obtained from the body muscles of the amphipod, *Orchestia platensis* Kr. and those of *Pleistophora ganapatii* from the gut of *Odontotermes horni* (Wasm.). The extrusion of the polar filament by

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a suitable method was taken as an indication of the viability of the spores. Air dried smears of the spores of *Pleistophora ganapatii* required the addition of a drop of saline and exertion of pressure to release the polar filaments in 90 percent of the spores while in *Thelohania orchestii* air dried smears required treatment with hydrogen peroxide for the release of the polar filaments. To test the viability of the spores at different temperatures the spores obtained from infected hosts were thoroughly washed in repeated changes of saline and kept at the required temperature for the desired length of time. The spores were kept in small embryo cups containing normal saline to which a few drops of 2.5 percent aqueous solution of potassium dichromate was added to prevent the growth of bacteria and ciliates. The spores in batches of not less than 100 each were removed at regular intervals and the percentage viability of the spores calculated. The spores obtained from a single host were used for experiments conducted to test the viability of the spores at any particular temperature. A sample of spores from each host was initially tested for their percentage viability before the commencement of the experiments to study the effect of temperature on the viability of the spores. Every experiment was repeated thrice before any conclusion was drawn.

Observations

Effect of Temperature on the Spores of *Thelohania orchestii*

Spores of *Thelohania orchestii* kept at a temperature of 25°C which is the temperature at which the hosts normally live lost their viability slowly. Thus 90 percent of the spores remained viable after 24 h, 80 percent after 48 h, 73 percent after 72 h and finally all the spores were killed in 14 days. At 12°C they remained viable for 124 h during which period there was a progressive reduction in percentage viability. At 8°C they were viable for 60 h and at 0°C for 48 h.

When the spores were kept at temperatures higher than 25°C they lost their viability rapidly. Thus the spores were viable for 78 h at 30°C, for 48 h at 35°C, 6 h at 37.5°C for 20 min at 40°C and were killed instantaneously at 42°C. The spores of *Thelohania orchestii* survive for the longest period at 25°C which corresponds to the temperature at which the host normally lives (Fig. 1).

In all the above experiments the heat applied to the spores should be considered as moist heat because they were always kept in a liquid medium. If the spores were air dried or kept in a dessicator they lost their viability within 24 h.

Effect of Temperature on the Spores of *Pleistophora ganapatii*

Spores of *Pleistophora ganapatii* which were subjected to a temperature of 0°C lost their viability at the end of 21 days. Spores maintained at 8°C lost their viability in 23 days and those at 12°C in 26 days. Spores

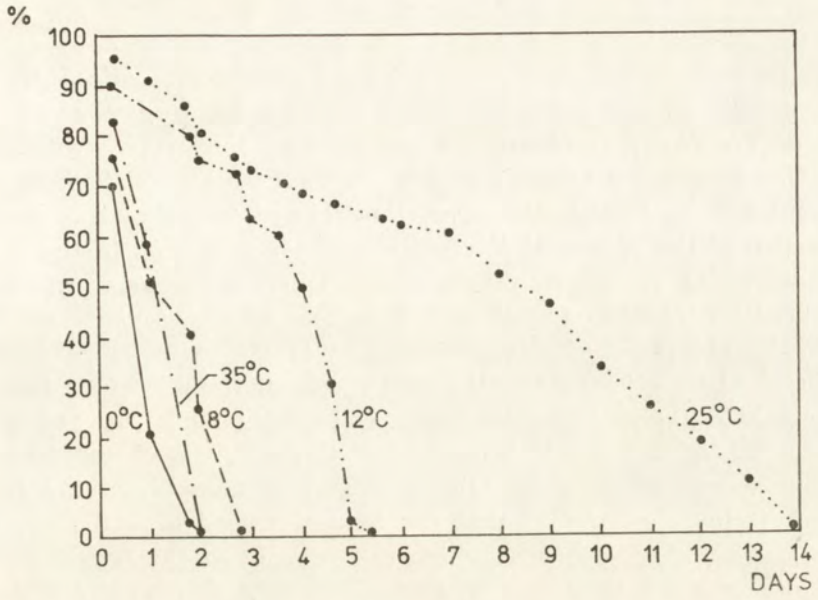

Fig. 1. Percentage viability of spores of *Thelohania orchestii* at different temperatures

subjected to 25°C which is the temperature at which the termite host normally lives remained alive for 76 days. The spores maintained at temperatures higher than 25°C lost their viability rapidly. Thus they are viable for 18 days at 30°C, 3 days at 37.5°C and death was instantaneous at 40°C. The data in Fig. 2 shows the percentage viability of the spores at each temperature. Spores kept at a temperature at 25°C were viable for the longest period which is also the ambient temperature of the termite hosts.

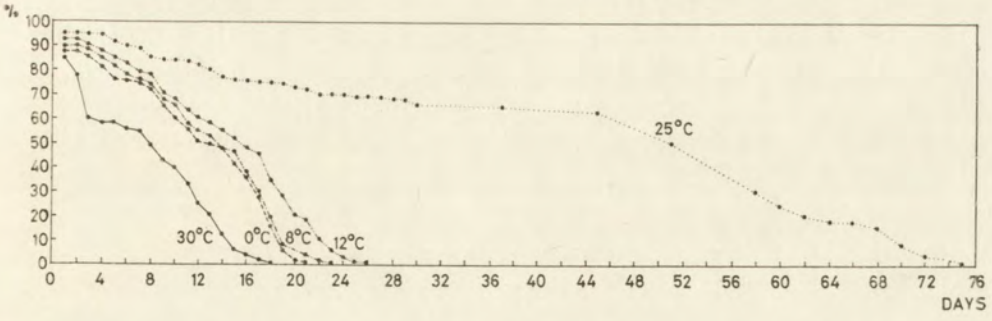

Fig. 2. Percentage viability of spores of *Pleistophora ganapatii* at different temperatures

Discussion

In the present series of experiments it has been noticed that the spores of *Pleistophora ganapatii* remained viable for 76 days at 25°C and those of *Thelohania orchestii* for 14 days at 25°C. They were killed instantaneously when exposed to 42°C in the case of *Pleistophora ganapatii* and 40°C in *Thelohania orchestii*. Allen and Brunson (1947) observed that the spores of *Nosema destructor* occurring in the eggs of *Gnorimoschema* sp. became non viable when they were exposed to a temperature of 47°C for 20 min but those of *Pleistophora california* remained viable even after exposing them to the same temperature for 96 min. Allen (1954) states that the spores of the microsporidian such as *Thelohania hyphantrae* were viable at higher temperatures of 60° and 70°C for 20 min. Thus it is seen that the survival of the spores of different species of microsporidians varies considerably. In the present instance it has been found that the longest period of viability of the spores was at a temperature which corresponds to the temperature of the habitat of the host.

The lethal effect of higher temperature on the microsporidian spores and the developmental stages of the parasite as a control measure of microsporidiosis has been studied by several workers. Pojarkov (1940) observed that by exposing the pupae of the silk-worm moth to 32–34°C. *Nosema bombycis* can be checked. Similarly Lotmar (1944) has shown that the development of *Nosema apis* can be checked by keeping the bees at 37°C for 10 days. Pojarkov (1954) stated that the pupae kept for 16 h at 34°C and for 8 h at 21°C in the course of a day also killed the parasite. Similar studies in regard to the microsporidian parasite from the termite have not been possible because the termite hosts are very sensitive to changes of temperatures and they die quickly even at the room temperature of 30°C. Tropical termites are known to survive at 20–22°C but the temperature between 25–28°C in general is most favourable for breeding and testing of tropical termites (Krishna and Weesner 1969). Attempts to defaunate the amphipod hosts of the microsporidian parasites by exposing them to temperatures of 30°C and 35°C did not proove successful and temperatures above 35°C killed the hosts in about 30 min.

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RÉSUMÉ

La température optimum pour la viabilité des spores de *Thelohania orchestii* et *Pleistophora ganapatii* fut trouvés être 25°C. Les spores perdirent rapidement leur viabilité à des températures plus élevées et la mort fut instantanée à 42°C pour les *Thelohania orchestii* et à 40°C pour les *Pleistophora ganapatii*. À de basses température (eutre 12°C et 0°C) elles perdirent leutement leur viabilité et les spores furent complètement non-viables en 2 jours dans le cas des *Thelohania orchestii* et eu 21 jours dans le cas des *Pleistophora ganapatii* quand elles furent exposées à 0°C.

REFERENCES

Allen H. W. 1954: *Nosema* disease of *Gnorimoschema operculella* (Zeller) and *Macrocentrus ancylivorus*. Ann. Ent. Soc. America, 47, 407–424.

Allen H. W. and Brunson M. 1947: Control of *Nosema* disease of potato tuber worms a host used in the mass production of *Macrocentrus ancylivorus*. Science, 105, 394.

Astaurov B. L. 1956: Biologičeskoje dejstvije vysokih temperatur i prishiznenoje termičeskoje obezzaraživnoje. Infektionnye i Protozoinyje Bolezni Poleznyh i Vred. Nasekomyh. Moskva, 63–93.

Finney G. L., Flanders S. E. and Smith H. S. 1947: Mass culture of *Macrocentrus ancylivorus* and its host, the potato tuber moth. Hilgardia, 17, 437–483.

Kalavati C. 1976 a: The morphology and life-history of a new microsporidian parasite, *Thelohania orchestii* n. sp. from the muscles of an amphipod, *Orchestia platensis* Kr. and experimental infection. Acta Protozool., 15, 293–300.

Kalavati C. 1976 b: Four new species of microsporidians from termites. Acta Protozool., 15 (in press).

Karmo E. and Morgenthaler. O. 1939: The development of *Nosema apis* at various temperatures. Bee World, 20, 57–58.

Krishna K. and Weesner F. M. 1969: Biology of Termites. Vol. I. Academic Press, New York.

Lotmar R. 1944: Über den Einfluss der Temperatur auf den parasiten *Nosema apis*. Schweiz Bienner Zfg., 67, 17–19.

Ovanesjan T. T. and Lobzandze V. I. 1956: Prishiznenoje obezzaraživanje, oplodotvorenog pebrinoznoj greny tutovogo selkoprjada s pomoštšju kratkovremennyh progrevov gorjatščej vodoj. Infektionnye i Protozoinyje Bolezni Poleznyh i Vred. Nasekomyh, 177–189.

Ovanesjan T. T. and Lobzandze V. I. 1958: Pervye resultaty opytov po termičeskomu obezzaraživaniju pebrinoznoj greny tutuvogo selkoprjada kratkovremennym progrevom v gorjačej vode. Trudy. Inst. Morfologii Zivotnych. An SSSR 21, 184–215.

Pojarkov E. F. 1940: Selkovodstvo, Moskwa, Selchozgiz, 87 pp.

Pojarkov E. F. 1954: Biologičeskij metod borby s pebrinoj tutovogo selkoprjada na faze greny i kukolki. Infektionnyje i Protozojnyje Bolezni Poleznyh i Vred. Nasekomyh, 108–129.
Steinhaus E. A. and Hughes K. M. 1949: Two newly described species of microsporidia from potato tube worm, *Gnorimoschema operculella*. J. Parasitol., 35, 67–74.
Weiser J. 1961: Die Mikrosporidian als Parasiten der Insekten. Monogr. angew. Entomol., 17, Parey, Hamburg und Berlin, 149 pp.

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Effect of pH on *Blepharisma intermedium*. 5. Studies on Esterases

Synopsis. Esterases of *Blepharisma intermedium* grown in buffered media of different pH values and in unbuffered media have been characterized. Selective inhibition studies indicate six carboxylesterases, two arylesterases and one cholinesterase in all cases. Although the type and number of the enzymes remain constant, there are variations in their inhibitor sensitivities.

Esterases of ciliated protozoa have been studied to a considerable extent. These enzymes are said to have a catabolic role in vacuolar digestion (Parker 1968) and their presence seems to play a role in ciliary movement (Seaman and Houlihan 1951). It is also postulated that they are involved in synthetic reactions in ectoplasmic structures (Parker 1968).

Much of the work that has been carried out on the esterases of protozoa is on *Tetrahymena*. A number of nonspecific esterases have been shown to be associated with cytoplasmic granules and food vacuoles of *Tetrahymena* (Allen 1958). The esterase activity was found to increase with the aging of the culture in *Tetrahymena pyriformis* (Fennell and Pastor 1958, Koehler and Fennell 1964), and the distribution pattern of the aliesterases has been shown to depend on the stage of the growth cycle (Allen 1958, Koehler and Fennell 1964). Koehler and Fennell (1964) have used specific esterase inhibitors to separate and identify esterases of *Tetrahymena* by means of starch gel electrophoretic technique.

There are not many reports on the esterases of *Blepharisma*. Nonspecific esterases have been demonstrated by cytochemical methods in the infraciliary complex and cytoplasm of whole and regenerating cells of *Blepharisma intermedium* by Parker (1968). He has also reported the presence of lipase activity in the organism.

Esterases from various sources have been characterized by using differential inhibitor sensitivities (Bersohn et al. 1966, Forster et al. 1959 and Myers et al. 1957). This technique, which has not so far

been employed in the study of esterases of protozoans is employed to characterize the esterases of *B. intermedium*.

Material and Methods

Organisms grown in hay infusion media, and in citrate (pH 5.0 and 6.0) and acetate buffered media (pH 5.0 and 5.6) were used in the present investigation. Culture conditions were as described in our earlier communications (Shadaksharaswamy and Jyothy 1973, Kasturi Bai et al. 1969). Organisms in unbuffered hay infusion media are referred to as control in the text.

Enzyme Preparation

Organisms grown under the various growth conditions were harvested by low speed centrifugation (275 × g). The pellet obtained was repeatedly washed with distilled water. The packed organisms were homogenized in distilled water in an all glass Potter-Elvehjem type homogenizer surrounded by ice and salt mixture. The homogenate was centrifuged at 18 000 × g for 30 min. Protein determinations were made on the supernatant by the method of Lowry et al. (1951) and it was diluted suitably and used for assay. The enzyme preparation was used within 24 h although it was stable for over a week at 0°C.

Substrates and Inhibitors

The substrates used were 1-naphthyl esters of acetate (NA), propionate (NP) and butyrate (NB), obtained from Sigma. The inhibitors dichlorvos (2,2-dichloro--vinyl-dimethyl-phosphate), parathion (diethyl p-nitrophenyl thiophosphate) and carbaryl (1-naphthyl N-methyl carbamate) were purchased from Chemservice Corporation. Eserine sulphate was got from Sigma and PCMB (p-chloro mercuribenzoate) from Fluka.

Assay Procedure

Esterase activity was measured quantitatively by the colorimetric technique of Gomori (1953) as modified by van Asperen (1962). A stock solution of the substrate (3×10^{-2} M) was prepared in acetone. This was buffered with 0.034 M phosphate buffer at pH 7.0 by diluting 1.0 ml of the stock solution to 100 ml with the buffer, to give a substrate concentration of 3×10^{-4} M 0.2 ml of the enzyme was incubated with 1.0 ml of the inhibitor for 30 min at 25°C. To this 5.0 ml of the buffered substrate was added and the tubes were incubated at the same temperature for 30 min. At the end of the incubation period 1.0 ml of the colour reagent (a mixture of 5% sodium lauryl sulphate and 1% diazo blue B in the ratio 2.5 : 1) was then added both to stop the reaction and for the development of colour. The tubes were allowed to stand for 15 min and the optical density was determined at 600 nm.

To determine the optimum pH, the following buffers of 0.034 M concentration were used: acetate, phosphate and pyrophosphate. The pH range obtained was 5.0 to 9.0. 1-naphthyl acetate was used as the substrate in these experiments.

Results

Substrate Specificity

Activities of the esterases of *B. intermedium* towards the three 1-naphthyl esters used in this study and their K_m values are given in Table 1. The activities are expressed as μg 1-naphthol/100 mg protein/minute. NB was hydrolyzed at a higher rate than NP and NA.

Table 1

Hydrolysis of 1-naphthyl Esters by *Blepharisma intermedium* Homogenate

Substrate	Activity Units*/100mg protein	K_m (M)
1-naphthyl acetate	13.24	4.0×10^{-4}
1-naphthyl propionate	14.90	3.62×10^{-4}
1-naphthyl butyrate	18.48	2.94×10^{-4}

* 1 unit of activity is 10 μg product/minute.

Inhibitor Specificity

Inhibition patterns of the esterases of *B. intermedium* have been presented in Figs. 1 *1–5*. Plots of pI (negative $\log_{10}$ of M inhibitor concentration) vs. percent inhibition have been used to differentiate esterases (Aldridge 1953, Carino and Montgomery 1968, Montgomery et al. 1968 and Norgaard and Montgomery 1968). These plots enable to differentiate esterases which have different inhibitor sensitivities and are present in the same preparation hydrolyzing the same substrate. Myers et al. (1957) in their work on the esterases of mycobacteria obtained double sigmoid curves suggesting the presence of two esterases, which had different inhibitor sensitivities but hydrolyzing the same substrate. In these curves a plateau was reached where increasing amounts of inhibitor had little effect until sufficient inhibitor was present to start inhibiting the least sensitive esterase. However, enzymes with similar inhibitor and substrate specificities cannot be differentiated by this method.

Figure 1 *1*(a–e) shows the effect of inhibitors between pI values 2 to 8 on the esterase activity of *B. intermedium* grown in unbuffered media (control). With eserine sulphate, concentration corresponding to pI 2 could not be used because of its interference with the colour development. The dichlorvos inhibition curve (Fig. 1 *1* a) shows the presence of at least

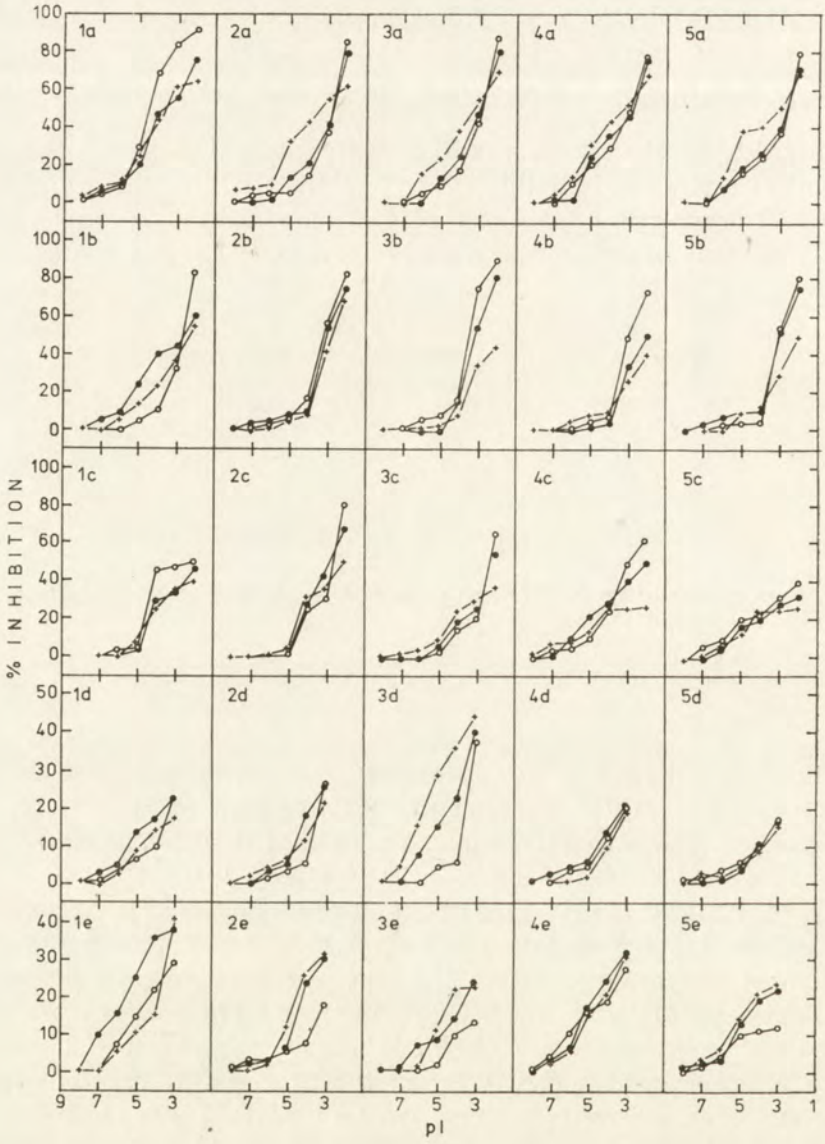

Fig. 1—5. *1* — Inhibition curves for the hydrolysis of 1-naphthyl esters by homogenate of *B. intermedium* (control) *2* — Inhibition curves for the hydrolysis of 1-naphthyl esters by homogenate of *B. intermedium* grown in acetate buffered media, pH 5.0 *3* — Inhibition curves for the hydrolysis of 1-naphthyl esters by homogenate of *B. intermedium* grown in acetate buffered media, pH 5.6. *4* — Inhibition curves for the hydrolysis of 1-naphthyl esters by homogenate of *B. intermedium* grown in citrate buffered media, pH 5.0. *5* — Inhibition curves for the hydrolysis of 1-naphthyl esters by homogenate of *B. intermedium* grown in citrate buffered media, pH 6.0.

Common to Figures 1—5: (a) — Inhibition with dichlorvos, (b) — Inhibition with parathion, (c) — Inhibition with PCMB, (d) — Inhibition with eserine sulphate, (e) — Inhibition with carbaryl. Percent inhibition is plotted against the negative $\log_{10}$ of the molar inhibitor concentration (pI), x- - -x — 1-naphthyl acetate, ●- - -● 1-naphthyl propionate, o- - -o 1-naphthyl butyrate

four enzymes. There is one enzyme between pI 4 and 6 accounting for about 64% NB hydrolyzing activity. The second enzyme is indicated between pI 3 and 4. The difference in inhibition between pI 2 and 3 among the three substrates indicates the presence of the third esterase. This enzyme is more active towards NP. The presence of considerable activity at pI 2 indicates a fourth enzyme which could be classified as a dichlorvos resistant enzyme.

The inhibition pattern with parathion is shown in Fig. 1 *1* b. This also suggests the presence of four enzymes. One between pI 4 and 6, the second between pI 3 and 4 and a third between pI 2 and 3. The second and the third enzymes have greater NB hydrolyzing activity, unlike the first which is more active towards NP. The fourth enzyme indicated at pI 2 is more resistant to parathion than dichlorvos.

The inhibition pattern with PCMB is shown in Fig. 1 *1* c. The enzyme between pI 4 and 6 shows preferential NB hydrolyzing activity and the second enzyme between pI 2 and 4 hydrolyses NP and NA to a greater extent.

Figures 1 *1* d and e show the inhibition pattern with eserine sulphate and carbaryl respectively. Both Figures indicate the presence of two enzymes. One between pI 3 and 4 and the other between pI 4 and 8. The extent of inhibition with both these carbamates is lower when compared with the other two inhibitors. Figure 1 *2*(a–e) show inhibition pattern of the esterases of the organisms grown in acetate buffered media of pH 5.0. Inhibition with different concentrations of dichlorvos (Fig. 1 *2* a) reveals four enzymes. The enzyme between pI 4 and 6 is more active towards NA and the activity decreases with NP and NB. This order of activity changes with the enzyme indicated between pI 3 and 4 (NB > NP > NA). Between pI 2 and 3 the hydrolysis of NB and NP is sensitive to inhibition but that of NA is quite resistant. A resistant enzyme at pI 2 indicates the fourth enzyme. The data presented in Fig. 1 *2* b shows that the major esterase activity is inhibited between pI 2 and 4. Two esterases are indicated in this region, one between pI 2 and 3 another between pI 3 and 4. The third esterase was inhibited between pI 4 and 6. Similar to control there can be a resistant enzyme at pI 2, as nearly 20% activity is not inhibited. The PCMB inhibition pattern in Fig. 1 *2* c shows the presence of two enzymes. The enzyme between pI 4 and 6 is equally active towards all the three naphthyl esters and a second enzyme between pI 2 and 3 has greater NB hydrolyzing activity. Figures 1 *2* d, e show the presence of two enzymes, between pI 3 and 8 of the carbamates.

Dichlorvos inhibition pattern of the esterases of the organism grown in acetate buffered media, pH 5.6, is given in Fig. 1 *3* a, indicating the

presence of four enzymes between the same pI values. Figure 1 *3* b also reveals four enzymes: Three between pI 2 and 6 and another at pI 2. PCMB inhibition pattern (Fig. 1 *3* c) shows one enzyme between pI 4 and 6 and another between pI 2 and 3. The pattern with carbamates (Fig. 1 *3* d, e) show two enzymes each, between pI values 4 and 8 and 3 and 4.

The number of enzymes indicated in the inhibition pattern of the organisms grown in citrate buffered media pH 5.0 and 6.0 (Figs. 1 *4* a–e and 5 a–e) are the same as those obtained with organisms grown in acetate buffers, except in the case of eserine sulphate.

Discussion

Esterases consist of a heterogeneous family of enzymes. Several workers (Augustinsson 1961, Guss and Krysan 1972, Mounter and Whittaker 1953 and Sinanuvong et al. 1971) have used selective inhibition by organophosphates and carbamates as a criteria to classify these hydrolases as: carboxylesterases, arylesterases and cholinesterases. Carboxylesterases (carboxylester hydrolases EC 3.1.1.1), formerly known as aliesterases or B-esterases, hydrolyze both aliphatic and aryl esters but not choline esters. They are inhibited by most organophosphorus inhibitors but not by carbamates. Arylesterases (arylester hydrolases EC 3.1.1.2) formerly known as A-esterases, are not inhibited by organophosphates or carbamates but are inhibited by PCMB. These enzymes hydrolyze aromatic esters, but normally not aliphatic esters. Cholinesterases (acetylcholine acetyl hydrolases EC 3.1.1.7 and acylcholine acyl hydrolases EC 3.1.1.8) are inhibited by both organophosphates and carbamate inhibitors.

Animal (Augustinsson 1961 and Forster et al. 1959), plant (Carino and Montgomery 1968, Norgaard and Montgomery 1968), insect (Sinanuvong et al. 1971) and protozoal (Koehler and Fennell 1964) esterases have been studied with regard to substrate and inhibitor specificities. Employing the same techniques, the present studies have revealed the existence of carboxyl, aryl and cholinesterases in *B. intermedium* grown under different pH conditions.

Comparison of the inhibition pattern obtained with the organophosphorus inhibitors (dichlorvos and parathion) reveals the presence of six enzymes in the organisms grown in unbuffered media (Fig. 1 *1* a, b). Three enzymes are shown to be present between the pI values 2 and 3, 3 and 4 and 4 and 6 in the dichlorvos inhibition pattern. These three

enzymes are different from those that are inhibited by parathion at the same concentrations. If the same enzyme were inhibited, then the percent inhibition at the plateau would be expected to be the same. At pI 4 it can be seen that there is greater inhibition (68%) of the butyryl ester with dichlorvos than with parathion (10%). This shows the presence of two enzymes in the region pI 4 and 6 which have different inhibitor sensitivities. The inhibition between pI values 3 and 4 is also not due to the same enzyme. Dichlorvos inhibits the hydrolysis of the three substrates to the same extent, while this is not so with parathion. Between pI 2 and 3, there is little inhibition with dichlorvos whereas there is nearly 50% inhibition with parathion of N_B hydrolysis indicating that the two enzymes are different. These six enzymes inhibited by the two organophosphates can be classified as carboxylesterases.

The same number of carboxylesterases are shown to be present in the organisms grown under different buffered conditions. However, they differ in their inhibitor sensitivities. For example, the inhibition of the NB hydrolyzing activity with dichlorvos between pI 2 and 3 is more than 30% under the buffered conditions while it is only 7% in the control. On the other hand, with parathion the extent of inhibition is less in buffered media compared to control. Such differences are also noticed between pI 3 and 4 and more distinctly between pI values 4 and 6. Further, it is observed that between pI 4 and 6, the enzymes of the organisms grown in buffered media are quite resistant to parathion unlike what is observed in the control.

Inhibition sensitivities of the esterases of the organisms grown at pH 5.0 differs from those grown at higher pH's in acetate and citrate buffered media. In the case of organisms grown in acetate buffered media, pH 5.6, the enzymes are inhibited to a greater extent by parathion (pI 3 and 4) than in those grown at pH 5.0. Similar effects are also noticed with the organisms grown in citrate buffered media of pH 5.0 and 6.0.

The absence of complete inhibition of esterases of organisms grown under different conditions at pI 2 with both organophosphates indicates the presence of esterolytic activity. Augustinsson (1961) has reported the presence of arylesterases which are inhibited by PCMB but not by organophosphates. The esterolytic activity noticed at pI 2 may be due to the presence of arylesterases. In the case of pea esterases, though there is inhibition by PCMB, Montgomery et al. (1968) have stated that arylesterases are absent because of the complete inhibition of the enzymes by organophosphates. They attribute the action of PCMB to non-specific reaction of this inhibitor with essential -SH groups. However, in the present investigation since the enzymes are not

completely inhibited by organophosphates and there is considerable inhibition with PCMB (80% in acetate buffer) the presence of arylesterases is apparent. The inhibition pattern with PCMB indicates the presence of two arylesterases in all cases, though there are some differences in the extent of inhibition between the buffered and unbuffered conditions.

A comparison of the inhibition pattern obtained with the carbamates (eserine sulphate and carbaryl) reveals the presence of two enzymes, one between pI 4 and 8 and the other between 3 and 4, under the various conditions of investigation. As cholinesterases are generally inhibited by eserine suphate at concentrations of 10^{-5} M and lower (Krisch 1971), the inhibition noticed between pI 3 and 4 may not be that of cholinesterase. Sudderuddin (1972, 1973) in his work on the esterases of the green peach aphid has shown that 10^{-7} M eserine sulphate blocks cholinesterase activity completely and therefore higher concentrations of the carbamates must be inhibiting carboxylesterases. Therefore the enzyme inhibited between pI 3 and 4 may be a carboxylesterase and the other inhibited between pI 4 and 8 is a cholinesterase although its activity with the three substrates is low.

Blepharisma intermedium therefore contains a complex system of ester hydrolases. Under all chosen conditions six carboxylesterases, two arylesterases and one cholinesterase are indicated. Although the type and number of esterases remain constant, there are variations in the inhibitor sensitivities of these enzymes. Substrate specificity studies have also shown that NB is preferentially hydrolyzed. Investigations on insect esterases carried out in our laboratory and with pea esterases (Norgaard and Montgomery 1968), have shown that propionyl esters are hydrolyzed at a greater rate than acetyl and butyryl esters.

In our studies on the effect of pH on *B. intermedium* (Shadaksharaswamy and Jyothy 1973 a, b, 1976) it has been shown that pH of the environment has considerable influence on some macromolecular constituents and activity of some enzymes. It has been pointed out that the changes noticed have been due to the differences in the rate and extent of transport of various buffer components of the medium across the cell membrane. However, the existence of the same number of different types of esterases in the organisms grown under varying conditions indicates their metabolic importance.

ZUSAMMENFASSUNG

Es wurden Esterasen von *Blepharisma intermedium*, die in Puffermedien mit verschiedenen pH-Werten sowie in nichtgepufferten Medien ge zuchtet wurden, charakterisiert. Aus Untersuchungen der selektiven Inhibition ergaben sich stets

sechs Karboxylesterasen, zwei Arylesterasen und eine Cholinesterase. Obwohl Art und Zahl der Enzyme konstant bleiben, sind die Inhibitor-Empfindlichkeiten veränderlich.

REFERENCES

Allen S. L. 1958: Cytochemical localization of enzymes in sexual strains of the Protozoan, *Tetrahymena pyriformis*. Anat. Rec., 131, 526–527.

Aldridge W. N. 1953: Serum esterases. 1. Two types of esterases (A and B) hydrolyzing p-nitrophenyl acetate, propionate and butyrate, and a method for their determination. Biochem. J., 53, 110–117.

van Asperen K. 1962: A study of housefly esterases by means of a sensitive colourimetric method. J. Insect Physiol., 8, 401–416.

Augustinsson K. B. 1961: Multiple forms of esterase in vertebrate blood plasma. Ann. N. Y. Acad. Sci., 94, 844.

Bersohn J., Barron K. D., Doolin P. E., Hess A. R. and Hedrick M. J. 1966: Sub-cellular localization of rat brain esterases. J. Histochem. Cytochem., 14, 455.

Carino L. A. and Montgomery M. W. 1968: Identification of some soluble esterases of the carrot (*Daucus carota* L.). Phytochem., 7, 1483–1490.

Fennell R. A. and Pastor E. P. 1958: Some observations on the esterases of *Tetrahymena pyriformis* W. I. Evidence for the existence of aliesterases. J. Morph., 103, 187–202.

Forster T. L., Bendixen H. A. and Montgomery M. W. 1959: Some esterases in cow milk. J. Dairy Sci., 42, 1903–1912.

Gomori G. 1953: Human esterases. J. Lab. Clin. Med., 42, 445–453.

Guss P. L. and Krysan J. L. 1972: Esterases and the identification of Lipases from eggs of *Diabrotica undecimpuntata* Howardi and *D. virgifera*. J. Insect. Physiol., 18, 1181–1195.

Kasturi Bai A. R., Srihari K., Shadaksharaswamy M. and Jyothy P. S. 1969: The effects of temperature on *Blepharisma intermedium*. J. Protozool., 16, 738–743.

Koehler L. D. and Fennell R. A. 1964: Histochemistry and Biochemistry of the polysaccharides, esterases and dehydrogenases of *Tetrahymena pyriformis* (Strain W.) J. Morph., 114, 209–223.

Krisch K. 1971: The Enzymes. (ed. Boyer P. D.) Academic Press Inc., New York and London, 5, 43.

Lowry O. H., Rosebrough N. J., Farr A. L. and Randall R. J. 1951: Protein measurement with the Folin phenol reagent. J. Biol. Chem., 193, 265–275.

Montgomery M. W., Norgaard M. J. and Veerabhadrappa P. S. 1968: Purification and substrate and inhibitor specificities of carboxylesterases of the pea (*Pisum sativum* L). Biochim. Biophys. Acta 167, 567–574.

Mounter L. A. and Whittaker V. P. 1953: Hydrolysis of esters of phenol by cholinesterases and other esterases. Biochem. J., 54, 551–559.

Myers D. K., Tol T. N. and De Jonge M. H. T. 1957: Aliesterases VI. Selective inhibitors of the esterases of brain and saprophytic mycobacteria. Biochem. J., 65, 232–241.

Norgaard M. J. and Montgomery M. W. 1968: Some esterases of the pea (*Pisum sativum* L). Biochim. Biophys. Acta 151, 587–596.

Parker J. W. 1968: A cytochemical investigation of the nuclear and enzymatic activities. Ph. D. Thesis, Stanford University, U.S.A.

Shadaksharaswamy M. and Jyothy P. S. 1973 a: Effect of pH on *Blepharisma intermedium* 1. Changes in fission rate and oxygen consumption. Acta Protozool., 12, 117–124.

Shadaksharaswamy M. and Jyothy P. S. 1973 a: Effect of pH on *Blepharisma intermedium* 2. Cytochemical changes. Ann. Histochim. 18, 149–158.

Shadaksharaswamy M. and Jyothy P. S. 1976: Effect of pH on *Blep-*

harisma intermedium 3. Changes in the levels of some macromolecules, free amino acid and protein pattern. Acta Protozool., 15, 57–65.

Seaman G. R. and Houlihan R. K. 1951: Enzyme systems in *Tetrahymena geleii* S: II. Acetylcholinesterase activity. Its relation to motility of the organism and the coordinated ciliary action in general. J. Cell Comp. Physiol., 37, 309.

Sinanuvong C., Knowles C. O. and Kearby W. H. 1971: Electrophoretic studies of certain hydrolases from the smaller European elm bark beetle *Scolytus multistriatus*. J. Kans. Entomol. Soc., 44, 408–413.

Sudderuddin K. I. 1972: Some biochemical and toxicological studies of organophosphate resistance in *Myzus persicae* Sulz. Ph. D. Thesis, University of London.

Sudderuddin K. I. 1973: An in vitro study of esterases, hydrolyzing nonspecific substrates, of an organophosphate resistant strain of the green peach aphid. *Myzus persicae* (Sulz). Comp. Biochem. Physiol., 44 B, 1067.

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Non-axial Cell Frame Movements and the Locomotion of *Amoeba proteus*

Synopsis. Principal types of movements, effected in the directions not corresponding to the main axis of locomotion, are described and classified as follows: side movements representing uniform lateral shifts of the whole cell axis, turning movements consisting in reorientation of the former motory axis and turning the cell at the spot as one block, bending movements effected by amoeba trunk or by individual pseudopodia. Non-axial movements may be performed as well in the horizontal plane as in the vertical direction. All the diversity of co-axial and non-axial movements of cell contours, i.e., in fact the whole cell locomotion, are explained by: the gradient of posterior contraction and anterior extension of the semi-rigid cell frame constituted by the cortex, migration forward of the attachment sites which are points of support for the contraction and extension forces, transmission of tensions by the cell frame itself. Intracellular streaming is considered to be a phenomenon largely independent from cell locomotion, and not its primary causal factor.

In the precedent paper (Grębecki 1976) the co-axial cell frame movements were described and their mechanism analyzed. Co-axial movements consist in active shifting of the whole cell periphery, usually forwards and in some cases backwards along the main axis of locomotion, what is reflected in changing the position of external contour of amoeba at the successive stages of its locomotion.

The first purpose of the present study was to demonstrate and to explain the capability of amoeba to perform non-axial movements, i.e., to change the position of the whole cell or of individual pseudopodia, in the directions independent from the main axis of locomotion and from the course of intracellular streaming. In the second part of this article an essay will be made to integrate all present and earlier data, accumulated by the author and his co-workers, on the motory behaviour of the semi-rigid frame forming the cell periphery, in order to incorporate them into a general concept of amoeba locomotion. It should be

added that, after the precedent papers were submitted to the press, two important studies proving the contractile capacities of amoeba cortex were published by Korohoda and Stockem (1975) and Kalisz and Korohoda (1976), which show that these authors, in spite of different techniques and approach, arrive to the similar view on the mechanism of amoeboid movement, as that developed recently in this laboratory.

Material and methods

The cultures of *Amoeba proteus* were grown in the Chalkley medium and fed on *Colpidium*. All the methods were exactly the same as used in the precedent study (Grębecki 1976). Three techniques of recording locomotion were used, as before: (1) analysis of superimposed cell contours, at the successive locomotion stages, selected from time lapse ciné-films turned with the frequency of 1/s, (2) photographic recording by double-exposure of a moving cell, on the same plate at the 20 s interval, and (3) photographic time-exposure recording by tracing on the plate the movement effected by the cell during 20 s. For the side-viewing, the vertical chamber mounted on the horizontal microscope, or the double mirror system, were used in the same way as in the precedent study.

Side Movements of Whole Cell

Examination of superimposed contours of successive locomotion stages of amoebae often provides examples of side movements of the whole cell. Such movements may be perpendicular to the principal axis of locomotion and to the main stream of endoplasm, and cannot be therefore attributed directly to the intracellular transport of material neither to the parallel co-axial crawling of the semi-rigid cell frame.

Figure 1 A presents the case of a specimen which, during 20 s of recording, preserved one older frontal pseudopodium gradually weakening but still actively progressing in the upper right direction, and produced second leading pseudopodium vigorously advancing to the upper left. Main stream of endoplasm in the contracting parts of its trunk was flowing from lower left to upper right on the picture. When comparing the contours of this amoeba in both 0"–10" and 10"–20" interstage periods one can easily discern, besides the changes expressing the anterior expansion and posterior contraction and besides the co-axial general crawling forwards, another basically different component: a clear shift of the whole contour to the right, in the direction nearly perpendicular to the principal axis of locomotion.

Another example, obtained by means of time-exposure technique,

is shown on the Pl. I 1. The main direction of streaks produced in this photogram is perpendicular, and not parallel, to the axis of locomotion of the animal recorded. That means that during the 20 s exposure time the whole cell performed an important side movement.

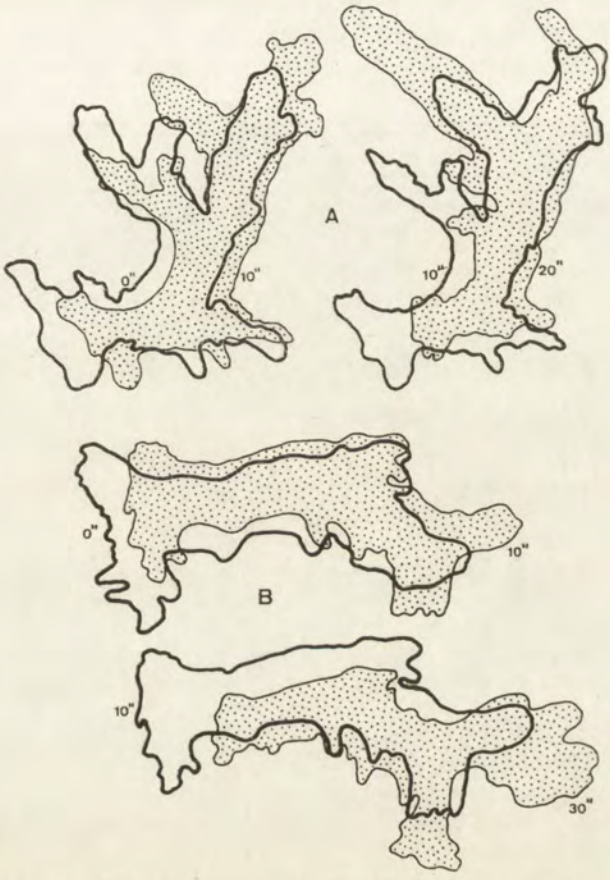


Fig. 1. Two examples of side movements. In all figures drawn in this convention, thick empty contours represent the initial stage of locomotion, and thin dotted profiles show the shape and position assumed by amoeba after a given time interval, as indicated in seconds

Side movements are far to be a constant component of cell locomotion in amoeba. All the examples given here were selected from much longer series of records, what means that the specimens in question, in earlier and later periods, manifested only the co-axial endoplasm streaming and the co-axial ectoplasm crawling, but no lateral drift.

Lateral component of locomotion may not only appear and disappear rather suddenly, but sometimes it can also unexpectedly change its direction. For instance, the specimen presented in the Fig. 1 B manifested

the presence of lateral component of its locomotion over a total period of 30 s, but for the first 10 s it has been directed to the left in respect to the principal axis of locomotion, and during the last 20 s the side movement was effected in the opposite, right direction.

There exist records which permit to draw some conclusions as to the possible mechanism of side movements. One type of mechanism is suggested by the Fig. 2 A. The principal axis of locomotion of the

Fig. 2. Side movements shown by superposing several contours of successive locomotion stages. Time corresponding to the first position and to the last stage of the sequence is given in seconds. Arrows indicate two lateral growing pseudopodia which push amoeba aside (in A), and one contracting pseudopodium which provokes a side movement by pulling the cell (in B)

specimen recorded was directed upward on the picture. However, two lateral growing pseudopodia were pointing out to the right. It is clearly seen that at the interval separating the stage 0" and the stage 20" they both increased in length more than twice each, but their tips hardly

effected any substantial advance on the substrate. So, in order to extend, they pushed the whole cell aside, at the angle of 90° in respect to its main axis of locomotion.

A similar example is brought by the Pl. I 2, obtained with the double-exposure method. Two different positions assumed by the amoeba at the interval of 20 s indicate that it has been pushed aside by the extending but non-advancing pseudopodium seen in the lower right corner of the picture.

Second type mechanism of side movements may be deduced from the Fig. 2 B. In the specimen presented here the main axis of locomotion was directed from down to the upper left of the field but a lateral contracting pseudopodium, pointing straight upwards on the picture, was kept firmly attached at its tip. So, when contracting, it pulled the whole cell, at the angle of 45° in respect to its principal axis of locomotion.

Both mechanisms described are not essentially different from one another: pushing aside by a lateral pseudopodium extending but not advancing, or pulling aside by a lateral pseudopodium contracting but not withdrawing. Since the blockade of progression of an extending pseudopodium, and of withdrawal of a contracting one, result from their firm distal attachment to the substrate, and because such pseudopodia need these attachment points to exert their respective pushing or pulling effects, the following general conclusion may be drawn: side movements appear when the attachment to the substrate is asymmetrical, being stronger at some lateral points than along the principal axis of locomotion.

This mechanism is easier detected in amoebae with elongated lateral pseudopodia (as those represented in the Fig. 2) but probably it applies equally to more compact polypodial forms (as in the Fig. 1) because of asymmetrical distribution of their supporting knobs and "ventral" pseudopodia, invisible in the horizontal plane but perfectly seen in side-view (Bell and Jeon 1963, Haberey 1971, Grębecki 1976).

Turning Movements

Most usual way of changing the direction of locomotion in amoeba consists in producing a series of new leading pseudopodia, modifying gradually the angle of advancing front. Specimen shown in the Fig. 3 changed its direction from upper left to the right on the picture, by extending frontal leading pseudopodia to upper left at the time 0", straight up and upper right at the time 10", to upper right at the time 20", to upper right and to the right at the time 30", and eventually straight to the right at the time 40". General feature of this type of

Fig. 3. Turning by successive modifications of direction in newly formed anterior segments of the main axis of locomotion

behaviour is that the axis of locomotion changes its direction step by step, only due to the addition of new differently oriented anterior segments, but without any reorientation of its preexistent older course.

However, there exists in *A. proteus* an essentially different manner of changing the direction, apparently not yet described in the literature, which consists in total reorientation of the preexisting axis of locomotion and results in that the whole cell turns as one block at the spot.

The individual shown on the Fig. 4 A moved during fist 10 s forward in the lower right direction, and simultaneously manifested a very distinct side movement to the right in respect to its axis (exactly in the same manner as specimens represented in the Fig. 1). During next 10 s its front ceased to advance and amoeba effected a bending movement. After that, during last 10 s it turned as a block, almost at 90° in the anti-clockwise direction, and assumed a new upper left orientation. The individual presented in the Fig. 4 B practically did not effectively locomote during 60 s but it manifested a changing pattern of non-axial movements: a very slight anti-clockwise turn during first 10 s, much more distinct clockwise turn at the interval 10"–20", then 10 s of rest

(only co-axial motion of the cell frame is seen), and finally an important and sustained turning in the clockwise direction lasting 30 s.

Plate II 3 brings a time-exposure record of this type of behaviour: the circular pattern of streaks reveals that the cell turned as one block during the 20 s of exposure.

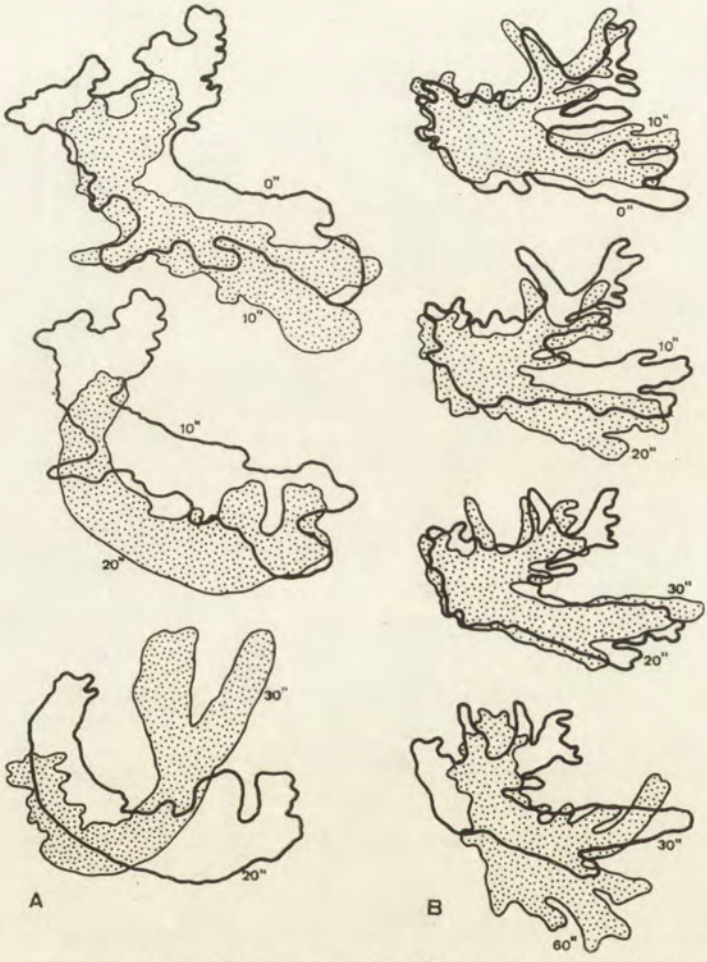


Fig. 4. Two examples of turning the whole cell as one block at the spot, with some earlier cell frame movements which preceded the decisive turn

The mechanism of turning by simultaneous reorientation of the whole axis of locomotion is probably similar to that of side movements (cf. Fig. 2): firm attachment of lateral pseudopodia which exert pulling effects if contracting or pushing effects when extending. Lateral pulling or pushing may result in a simple side movement as described before, only if the attachment along the main body axis is inexistent or easily

broken. Otherwise, if the lateral attachment has to compete with equally strong attachment zone situated in the central part of amoeba trunk, the resulting force vector becomes excentrically oriented in respect to the cell body. One can eventually conclude that the turning at the spot of the whole cell as one block appears when an asymmetrical lateral attachment is situated excentrically and interferes with equally stable attachment points along the principal axis of locomotion.

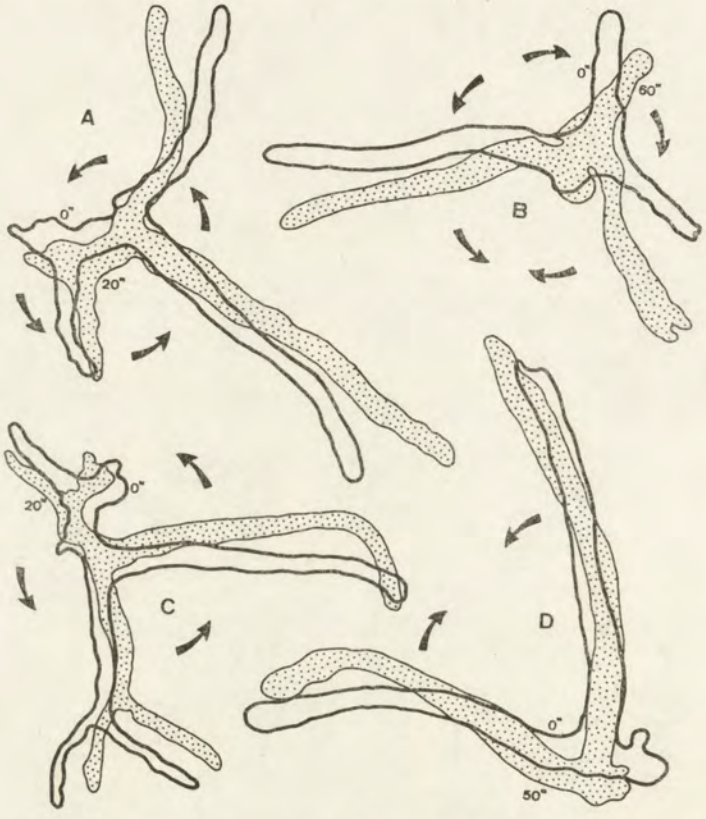


Fig. 5. Turning at the spot in amoebae with elongated thin pseudopodia. A and C: uniform turning of whole cells, B and D: turning complicated by independent movements of some pseudopodia

This explanation is indirectly supported by the fact that turnings at the spot are usually associated with other types of non-axial movements, and in particular with the side movements (cf. Fig. 4). It seems also probable that the same mechanism of asymmetrical and excentric attachment could explain rotations of amoeba cells gently pressed between two slides, phenomenon described by Czarska and Grębecki (1965).

Another indirect argument in favour of this interpretation comes from the fact that turning as one block is much more common in specimens with very elongated pseudopodia (forms said "radiate" by Bovee and Jahn 1973), in which the adhesion is less stable and the position of

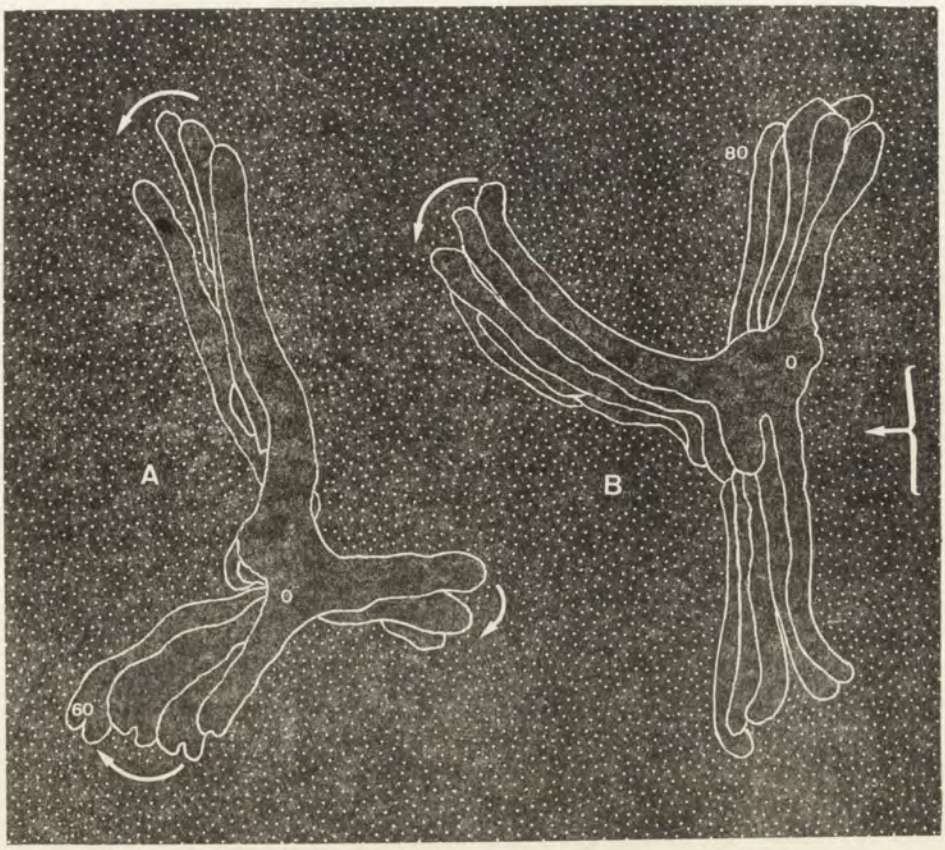


Fig. 6. Examples of non-axial pseudopodium movement combined with turning (A), and with a side movement (B) of the whole cell body

attachment points more variable in time and in space. Figure 5 A and C brings two examples of such amoebae, with long and thin pseudopodia, which turn as a block in the anti-clockwise direction. The behaviour of individuals presented in the Figs. 5 B and D, and 6 A and B, is more complex because the turning of whole cells is mixed with independent bending movements of some of their pseudopodia.

Bending of the Whole Body

Non-axial movements assume sometimes more complicated pattern, particularly that of bending the trunk and the whole body of amoeba. One can imagin at least three different mechanisms of bending.

First plausible reason of bending would be an asymmetry of cortical contraction on two opposite sides of the body axis. In such a case, the bending force should be exerted someway between the uroid and the middle region of trunk, i.e., in the posterior contracting 2/3 of amoeba (Grębecka and Grębecki 1975), provided that these regions are not firmly attached to the substrate. Behaviour of amoeba shown on the Fig. 7 A fulfills both requirements: positions of two contours separated by 10 s interval indicate that the attachment was stable only in the anterior region, and the maximal bending occured at the middle of the body length. Formation of a protuberance initiating a new pseudopodium, opposite to the maximal bending point, is another significant fact indicating the asymmetry of contraction–relaxation balance on both sides of the bending trunk. Another example of this type of bending, almost exactly similar in all details, is provided by the stage 10"–20" of amoeba shown in the Fig. 4 A.

Plate III 7 obtained by time-exposure photography presents a facultatively monopodial amoeba [1] attached at the middle-anterior region of its body (no movement of granules recorded in this area), with clear non-axial striation of the posterior part of its trunk, uroid and contracting pseudopodia. The striation reflects a bending movement of these regions, probably due to an asymmetrical contraction. Probably the same factor is responsible for bending commonly observed in permanently monopodial specimens [2] when their adhesion to the substrate is lacking or is unstable.

Second presumable mechanism of bending is an asymmetry of cortical extension on both sides of the body axis. Such an asymmetry may arise when the supply of unfolding cell membrane (Czarska and Grębecki 1966, Haberey et al. 1969) and/or the supply of extending ectoplasm material (Grębecki 1976), become restricted on one or another side of the main body axis. It is evident that the asymmetrical extension may be a decisive factor only in the anterior part of the cell.

Photographic recording provides many examples of such bending of

[1] The term "monopodial" is used here to describe specimens without the typical frontal cap, which only temporarily assume the monopodial shape and manifest unidirectional movement.

[2] The term "monopodial" is introduced in this case to designate individuals with stable morphology, bearing prominent frontal caps.

the anterior part of amoeba cell. Plate II 4 brings a time-exposure record and the Pl. III 5 a double-exposure picture, both showing uniform bending of all the frontal pseudopodia without any appreciable change of orientation in the trunk and in the uroid. Plate III 6 presents a facultatively monopodial amoeba which was apparently attached posteriorly and the bending was manifested by its free anterior part. More cases of this type of bending will be reported in the next chapter concerning the independent movements of pseudopodia.

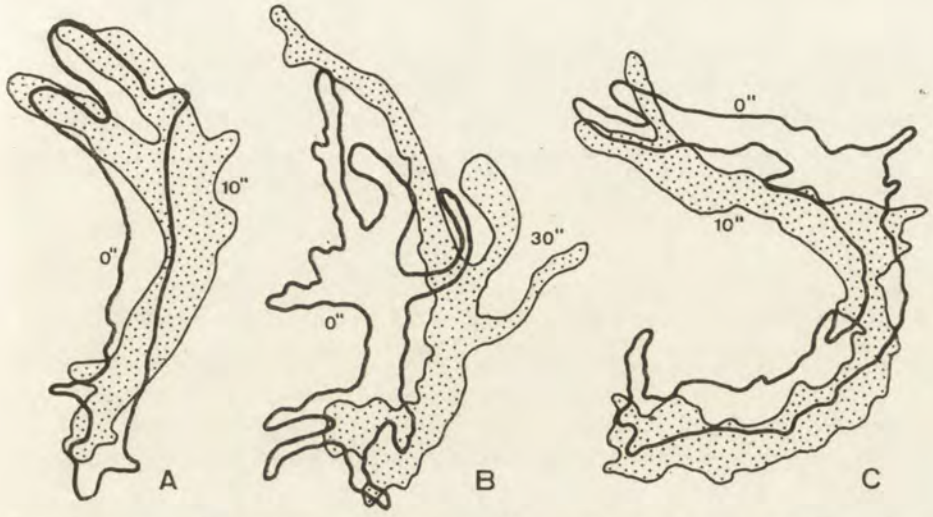


Fig. 7. Examples of bending in the trunk of amoeba: by asymmetrical contraction (A), by excessive extension between two fixed points (B), and by combined effects of different mechanisms (C)

Third possible mechanism of bending is an excessive extension between two fixed points. Excess of extension may be due to the cortical relaxation and/or to the inflow of endoplasmic material from pseudopodia situated laterally in the same or in different planes. The specimen shown on the Fig. 7 B changed its length considerably during 30 s, probably due to the inflow of material from contracting pseudopodia invisible in the filming plane. Nevertheless, its uroid as well as the tip of its frontal pseudopodium were keeping an almost stationary position. The result was an arch-like bending of the whole cell to the right.

Several mechanisms may act simultaneously and independently along the axis of one single specimen, what may produce more complicated results, as in the case presented in the Fig. 7 C. During 10 s the posterior part of this amoeba bent to the right whereas its anterior portion bent to the left, both contours forming intersection at the middle of their length.

Non-axial Movements of Pseudopodia

Non-axial movements of pseudopodia are particularly well seen in amoebae with long and thin pseudopodia. Such forms were used to obtain photographic time-exposure records presented in the Pl. IV and V, and the double-exposure pictures in the Pl. VI.

On the Pl. IV 10, 11, 12 and V 13 the oblique or tranversal striations reflecting the non-axial movements are seen as well as the initial and final contours of the respective pseudopodia. This means that the side movement is sometimes so quick and short that, during 20 s of exposure, the pseudopodium may be stationary at the beginning and its initial position is recorded as the first contour, then for a few seconds it moves aside producing streaks, and eventually it stops again and imprints the second contour corresponding to its final position.

First factor involved in side movements of pseudopodia may be any co-axial or non-axial motion of the whole cell frame including the pseudopodial basis. Such a case, as recorded in the Pl. V 16 by the time-exposure technique, may be easily discerned because the oblique striation is not limited to the pseudopodia themselves but it appears, in a parallel pattern, also in the adjacent portion of the trunk. On the contrary, the main tip and the lateral branch of pseudopodium shown in the Pl. V 15, manifest uniform but independent non-axial movements, because in the region between them there is no oblique striation at all.

Bending movements occur as well in contracting pseudopodia as in extending ones (cf. Fig. 8 A and B). Non-axial behaviour of contracting pseudopodia seems to have common basis with bending of the posterior part of trunk with the uroid: probably it results from an asymmetry of contraction on two sides of pseudopodium axis. The asymmetrical contraction may be either limited, in most cases to the pseudopodium basis, or generalized along the shaft. It is very characteristic of a local asymmetrical contraction that the pseudopodium moves aside as an apparently stiff shaft. Figure 8 B presents a case of contracting pseudopodium which, during 90 s, bent in this manner four times in different directions. Similar pattern is exhibited by the pseudopodium shown in the Fig. 9 at the 30"–70" period of recording.

On the contrary, when the asymmetrical contraction is generalized along the pseudopodium axis, it bends like a bow, i.e., the movement aside and arching follow the same direction. This kind of motion is seen in the Fig. 9 at the stage 70"–110".

Even temporarily inactive pseudopodia, manifesting neither advancing nor withdrawal during the recording time, may manifest as well the

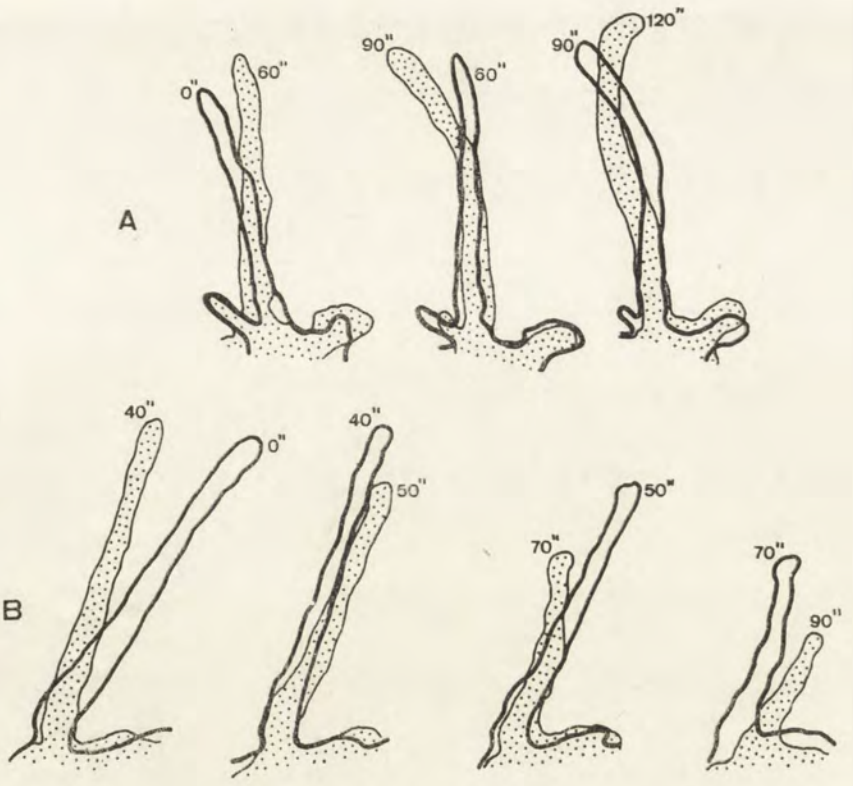


Fig. 8. Successive stages of bending of an advancing pseudopodium (A), and of a contracting one (B)

localized bending at their basis (Fig. 10 A) as the arching generalized all along their shaft (Fig. 10 B).

Non-axial movements of contracting pseudopodia assume more complicated patterns when different mechanisms are simultaneously acting together. In the example brought by the Fig. 9, the contracting pseudopodium was first slightly bending to the right at its basis, thereafter it bent in the same manner but more vigorously to the left, and eventually it arched to the right again. Moreover, in parallel with all these phases it was continuously drag to the right by a co-axial motion in the trunk. The result produced is a spectacular wave-like pattern of its motion.

The growing pseudopodia manifest non-axial types of motion essentially similar to the bending movements of the anterior part of the whole cell, as described before. In most cases their side movement is seen all along their axis (Pl. IV 8) or in their distal segments, but sometimes on the contrary, the tip manifests merely the regular co-axial behaviour and a non-axial movement is produced in the proximal part (Pl. IV 9).

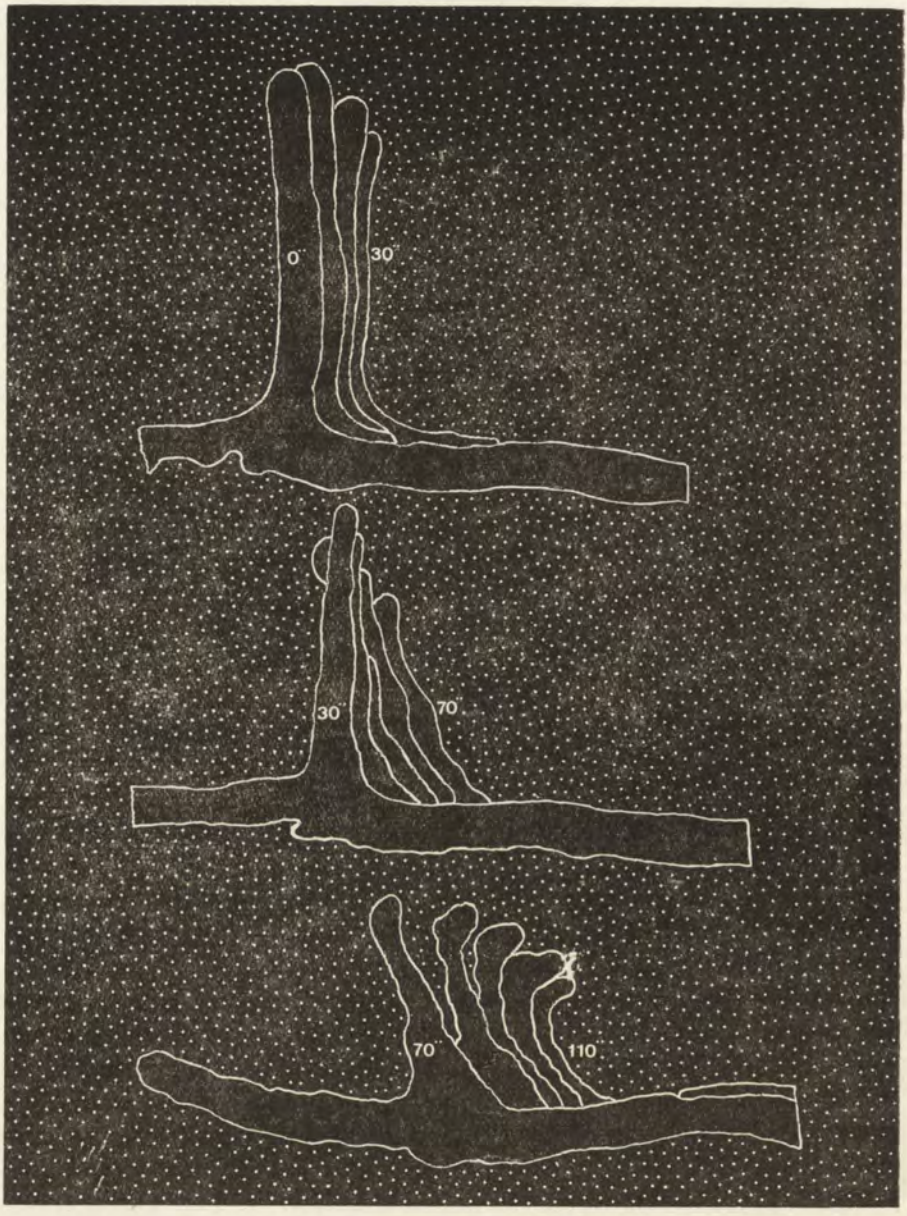


Fig. 9. Wave-like pattern of motion produced by a contracting pseudopodium, due to the alternating direction of its bending and to the simultaneous side drift of its basis

Most plausible explanation is that a growing pseudopodium may either deviate in its free portion situated in front of the attachment zone (Pl. IV 8) due to asymmetrical extension, either, when attached to the

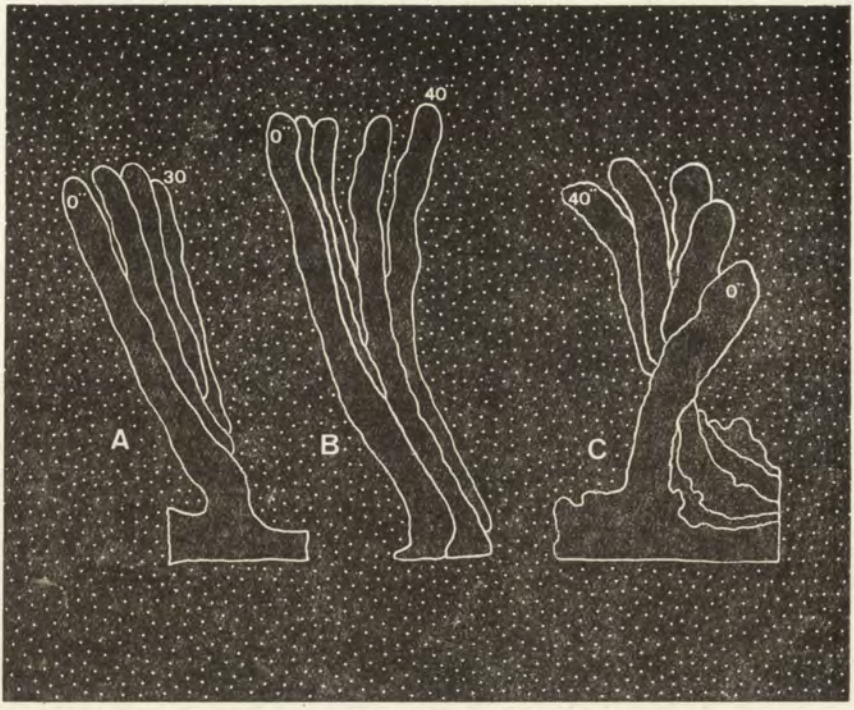


Fig. 10. Two examples of pseudopodia almost inactive axially but bending at the basis (A) or along all the shaft (B), and a growing pseudopodium with complicated bending pattern due to interaction of several mechanisms (C)

substrate someway at the middle of its length, it may bend in its proximal part due to an excessive extension between its basis and its attachment point (Pl. IV 9).

Bending of a growing pseudopodium due to an unilateral restriction of supply of the extended cortical material, sometimes may intervene only at the pseudopodium basis, as in the case shown in the Pl. VI 19. More commonly, however, the bending of growing pseudopodia seems to be uniform all along their axis which results (as in contracting pseudopodia) in parallel side movement and bow-like arching of pseudopodium shaft, as seen on the Pl. VI 17, 18 and 20.

In growing pseudopodia which are apparently attached to the substrate at the middle of their length, bending of the proximal segment due to an excessive extension between two fixed points, and bending of the distal segment due to an asymmetrical extension, may act simultaneously in opposite directions. This interpretation may explain rather common cases when growing pseudopodia bend in the S-like manner, and eventually their initial and final contours may intersect each other at their middle. Such a behaviour has been recorded by time-exposure

(Pl. V 14) and double-exposure photography (Pl. IV 21) as well as by time-lapse filming (Fig. 8 A stage 90"–120", and Fig. 10 C).

One should stress again that different mechanisms may interact and alternate in the same pseudopodium in the course of its extension. Slowly advancing pseudopodium shown in the Fig. 8 A was bending to the right at its basis only during 60 s, after that its distal part effected an arch-like bending to the left lasting 30 s, and during last 60 s its middle portion and its distal segment performed two opposite bendings and formed eventually intersection between the initial and the final contour. Another advancing pseudopodium presented in the Fig. 10 C produced a rather complicated pattern of 5 superimposed contours, separated by 10 s intervals, as result of a combination of bending to the left in its distal part and shifting to the right its basis together with a co-axial movement of the trunk.

Independent non-axial movements of pseudopodia may be sometimes confused with a turning of the whole cell, when two locomotory stages are compared separated by a long time interval. For instance, the individual shown on the Fig. 11 A apparently turned as a block over

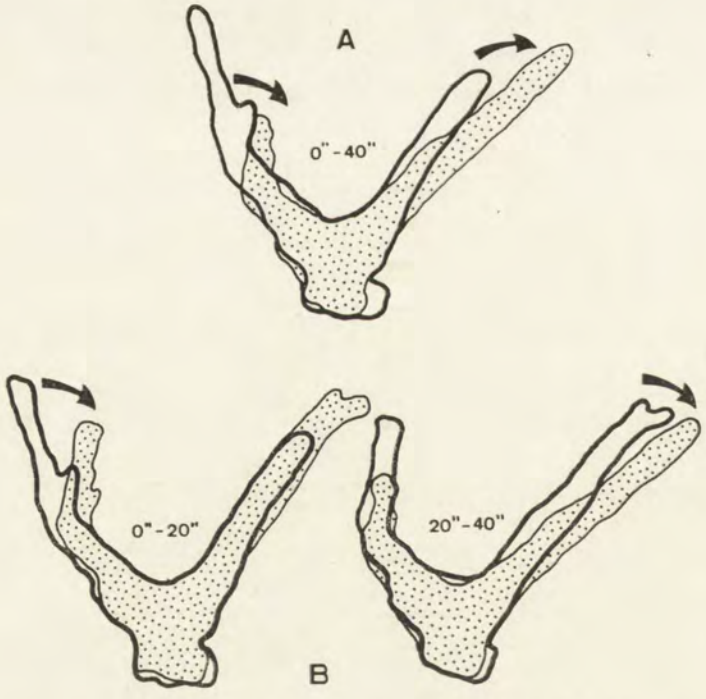


Fig. 11. Apparent turning of whole cell (A) which may be decomposed into two independent bending movements of separate pseudopodia (B)

the period of 40 s, but the Fig. 11 B puts in evidence that in fact during first 20 s only its left pseudopodium was bending when contracting, and during the next 20 s period the right pseudopodium effected a separate bending when advancing, both independent movements being directed to the right.

Vertical Component of Movement

The role of the position of attachment sites in producing the co-axial movements of the semi-rigid cell frame has been directly confirmed by viewing amoebae from the side (Grębecki 1976). It is impossible to collect a sufficiently large number of similar data for the non-axial movements, because they are effected at variable and hardly predictable angles in respect to the body axis. There is virtually no possibility to adjust the plane of side observation to the plane in which a non-axial movement is to be performed. Therefore, the results exposed in this chapter are limited to a demonstration that the semi-rigid cell frame of migrating amoeba displays a variety of non-axial movements in the vertical plane as well as it does it in the horizontal plane.

The vertical deviations from the principal axis of locomotion are even manifested in much more regular and sustained manner than the horizontal deviations rather scattered in time. The obvious reason is the presence of asymmetry factors, two at least, acting permanently in the vertical plane: cell-substrate interactions and the gravitation force (the role of this last factor is completely unexplored in amoeba but its influence could hardly be denied).

Very common type of vertical component in the locomotory behaviour is shown in the Fig. 12 A. Amoeba stops its former locomotion, for a certain time extends straight upwards, and then assumes the position described before by Bell and Jeon (1963) as "standing up" on the uroid. Soon later, an asymmetry appears in the upward expansion which leads to a critical change of position of the centre of mass. The cell drops down to one side and resumes normal locomotion in the new direction. This sequence of events may be considered as third possible manner of reorienting the axis of locomotion and turning of amoeba.

Much less common is an inverse sequence, as presented in the Fig. 12 B. Amoeba stops its forward movement and produces a reverse streaming of endoplasm. Its former leading pseudopodium looses contact with the substrate and retracts, what changes the equilibrium established before. Eventually the cell turns over to the back and adheres to the substrate with its former rear edge of the uroid.

Very common phenomenon in locomotion of polypodial amoeba is falling down of frontal free pseudopodia. Such pseudopodia usually extend forward and slightly upward until they bend down and establish contact with the substrate. Two examples of this type of vertical movement are given in the Fig. 13 A and B. This case falls to the cathegory of non-axial pseudopodial movements, and more precisely to that of their vertical bending, similar to their horizontal bending described in the precedent chapter, because it is not associated with any disequilibration of the main body.

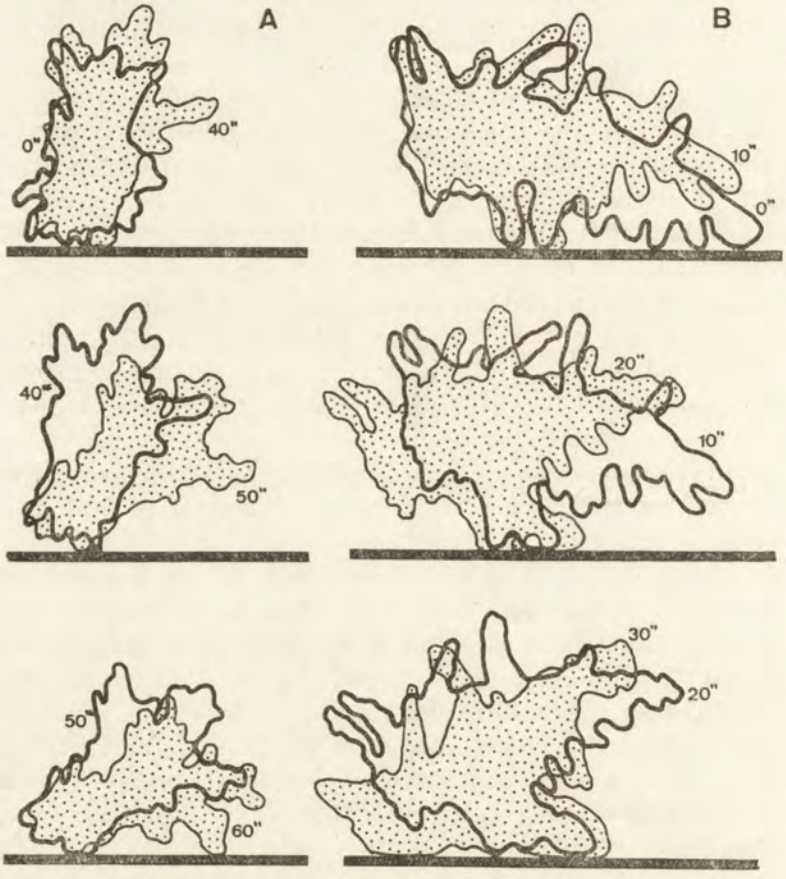


Fig. 12. Two cases of whole amoebae dropping to one side as result of a displacement of the centre of mass of cell body

The role of gravity in the vertical downward bending of free pseudopodia is still possible but not obviously necessary. It may be produced as well by the asymmetrical supply of membrane and cortical material, as in the case of horizontal bending of advancing pseudopodia. This

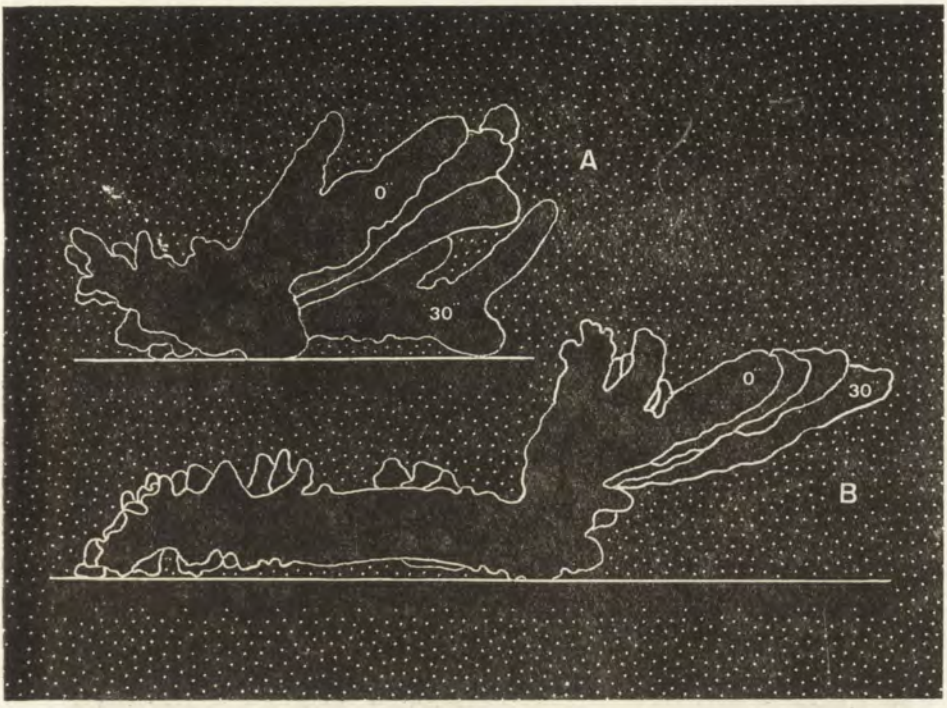


Fig. 13. Two examples of free frontal pseudopodia falling down

mechanism might act in vertical plane more regularly and effectively because the peripherial material needed for extension is easier available from the "dorsal" side of amoeba than from its "ventral" face where its supply is restricted by the attachment sites.

Second type vertical component of locomotion, regularly observed in strongly polypodial specimens, consists in lifting up the leading pseudopodia, or even the whole advancing front of the cell, depending on that how firm was the substrate attachment of the trunk. Some examples of this phenomenon are given in the Fig. 14 A and B and 15 A. In this case the side-viewing clearly indicates the underlying mechanism. A small supporting pseudopodium, forming a "ventral" branch of the leading frontal pseudopodium, not far behind its tip, starts to grow vigorously. It is attached at its own extremity to the substrate in the manner which prevents it from advancing. As a result, in order to extend it must wind up and push back the principal pseudopodium, or the whole advancing front if other attachment points permit.

The lifting up of the frontal pseudopodium is usually followed by its contraction when it is already repushed up and back. Then, the former supporting pseudopodium becomes the leading one, its tip starts

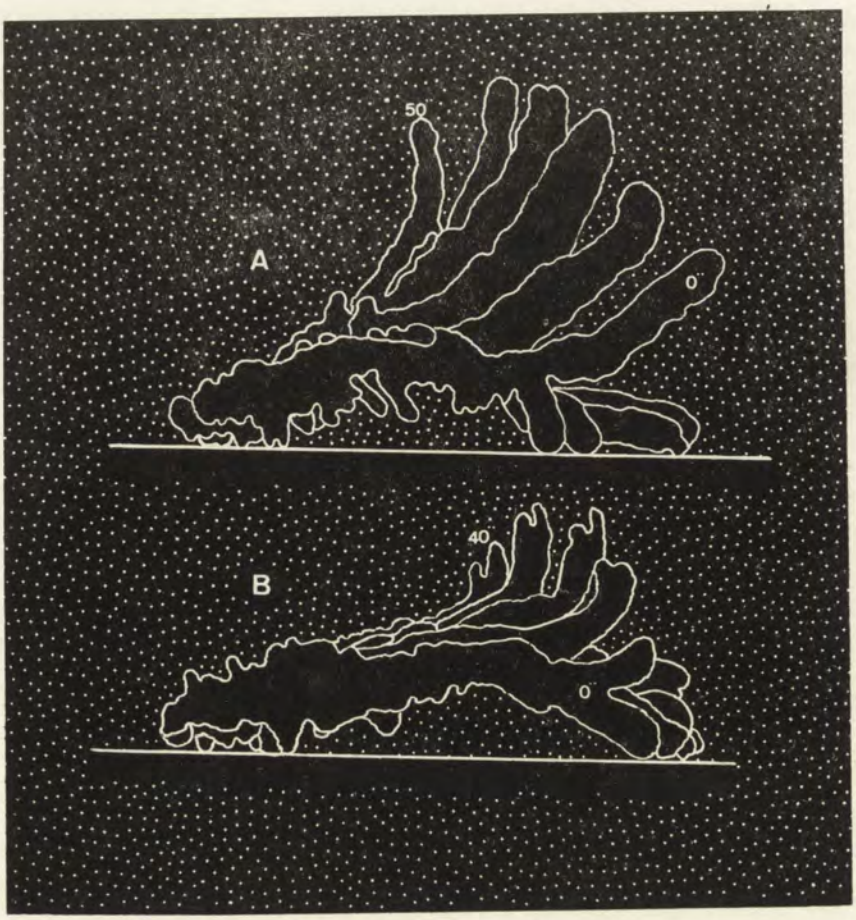


Fig. 14. Two examples of lifting up the whole frontal part of amoeba

advancing, and the whole sequence may repeat again when it will form its own "ventral" supporting knob.

It seems evident that the mechanism of lifting up the frontal pseudopodia is essentially similar to that of co-axial backward motion of the cell contour in amoebae firmly attached at their front, as analyzed in the precedent paper (Grębecki 1976), and to the mechanism of horizontal bending of frontal pseudopodia due to an excessive extension between two fixed points, as described earlier in the present study. It is even conceivable that in many instances all three phenomena represent simply the same movement, which we describe under its different aspects when looking at amoeba at different angles.

Lifting up of frontal pseudopodia may interfere during locomotion, and alternate, with their bending down and with other types of mo-

tion of the semi-rigid cell frame. The specimen shown as example in the Fig. 15 B was attached by the uroid, during first 20 s, and manifested a co-axial backward movement of its contour. Then, during next 40 s, attachment was established by its middle region what made possible normal locomotion with usual co-axial forward movement of the whole cell frame. As a result, at the end of this stage, amoeba lost its former equilibrium and in the following 30 s period it effected its first vertical movement falling over to the frontal side as one single

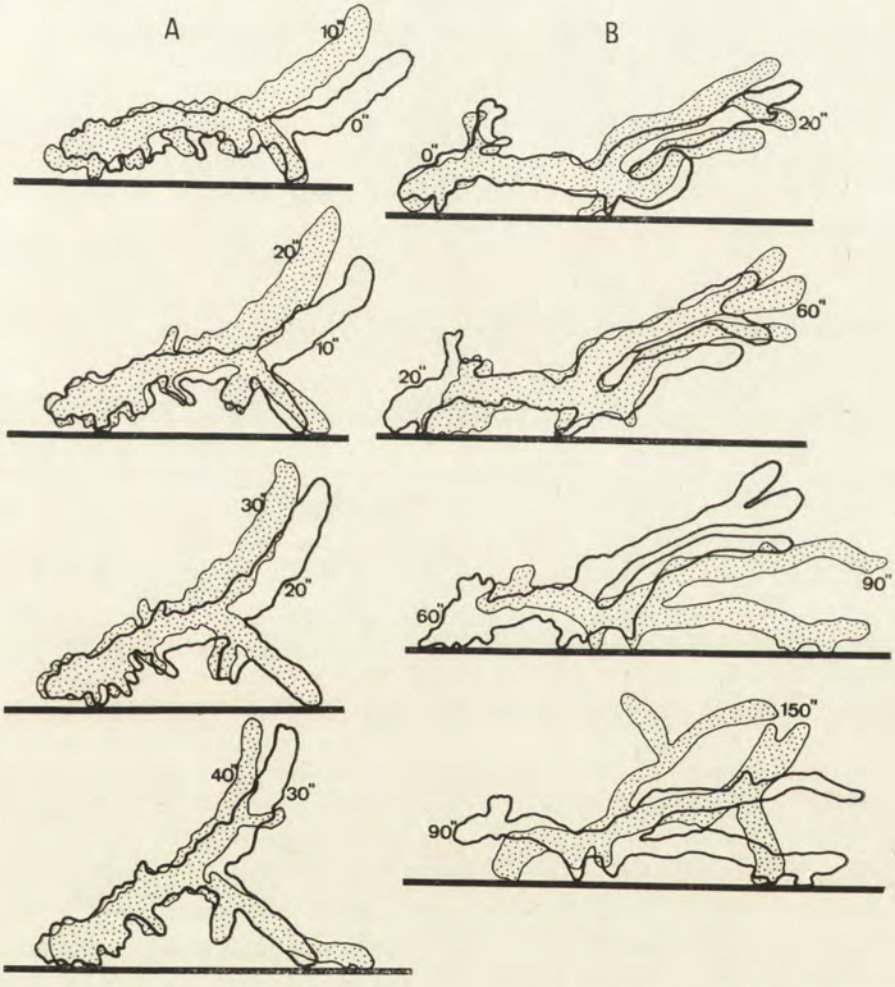


Fig. 15. Stage-by-stage analysis of lifting an amoeba up, from its frontal side, due to strong extension of a supporting pseudopodium (A), and of a more complex sequence of locomotion stages, with an alternation of falling down and lifting up phases, at the advancing front (B)

block: its anterior and central regions drop down with consequent lifting up the posterior part of trunk with uroid. Eventually, during last 60 s, the opposite vertical component interfered with the forward movement: lifting up the principal frontal pseudopodia by strong extension of a much smaller supporting pseudopodium.

Classification of Cell Frame Movements and their Factors

The purpose of this chapter is to summarize not only the observations described in this study, dealing with non-axial movements, but also those concerning co-axial movements which were analyzed in the precedent paper (G r ę b e c k i 1976).

In fact, just two basic factors and another subsidiary one, were sufficient in both studies to explain all the diversity of movements in question. The basic factors are: (1) posterior contraction — anterior extension gradient along the main axis of locomotion, as morphometrically demonstrated by G r ę b e c k a and G r ę b e c k i (1975), and (2) variable position of attachment sites along the axis of locomotion and/or out of the axis. The subsidiary factor invoked is a facultative asymmetry of contraction and/or extension on two opposite sides of the motory axis.

Various combinations of these few factors, which lead to different motory effects, are shown in the Table 1. In the following discussion reference to successive positions in the table will be given in brackets.

The simplest and most common situation (1) consists in contraction taking place in posterior 2/3 of cell body, extension of anterior 1/3 of it, and establishment of attachment zone, located symmetrically under the main axis of locomotion, nearly between the contracting and extending regions. Many such cases were examined in detail, and the general scheme of cell beviour was given in the Fig. 17, in the preceeding paper. The cell frame of such amoeba, in its posterior portion is pulled toward the attachment zone by contraction, and in its anterior segments it is pushed away from the attachment zone by extension. As a result, the whole cell frame moves coaxially forward in respect to the attachment site. This movement may perpetuate in illimited way in time and space, because new attachment points are being successively established more and more forwards along the motory axis. In the reality this scheme represents nothing else than a full description of normal locomotion of amoeba, which is rather peculiar in one respect: it might be done without any reference to the intracellular streaming.

With the same linear usual distribution of contraction, attachment and extension, the non-axial bending movements may sometimes com-

plicate the normal pattern of locomotion, when contraction and/or extension become asymmetrical on both sides of the principal axis of locomotion. In the case of an asymmetry of contraction intervening in the horizontal plane (2), the contracting structure lying behind the attachment zone (the whole posterior body part or a contracting pseudopodium) bends laterally. If the asymmetry of contraction is vertical (3), the posterior part of trunk with uroid may be lifted up or brought down, as shown in the Fig. 3 in the earlier paper. When the extension happens to be horizontally asymmetrical (4), the whole anterior cell portion in front of the attachment zone, or free advancing pseudopodia alone, manifest lateral bending. With the extension being asymmetrical in the vertical plane (5), the free advancing pseudopodia would as a rule bend towards the substrate.

Asymmetry of contraction, which is an active phenomenon, is easily conceivable, but the asymmetry of extension, which is based on the relaxation usually thought to be passive, needs some explanation. Elongation of extending anterior regions may become asymmetrical if a reserve of extended material is not equally easy available on both sides of the body axis. Similar phenomenon has been shown by Czarska and Grębecki (1966) in some cases of uneven distribution of folded membrane, which resulted in its asymmetrical unfolding over advancing pseudopodium. The same mechanism may apply well not only to the cell membrane but to deeper layers of the extending semi-rigid frame. Extension of leading pseudopodium may become horizontally asymmetrical, when the supply of cortical material becomes restricted on one side by competition of another pseudopodium extending laterally. In the vertical plane, it is regularly restricted on the "ventral" side by the sites of attachment to the substrate.[3] Probably for that last reason leading pseudopodia bend towards the substrate even against gravity (in specimens locomoting upside down under the slide, in a hanging drop).

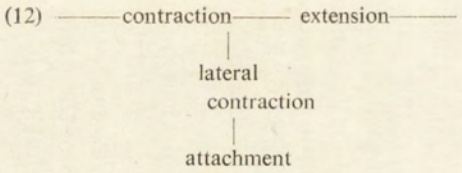
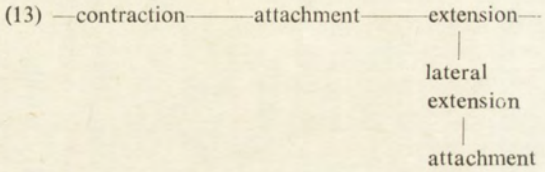
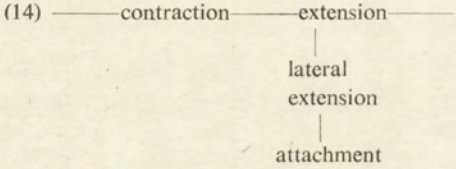
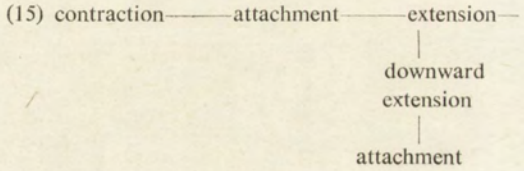
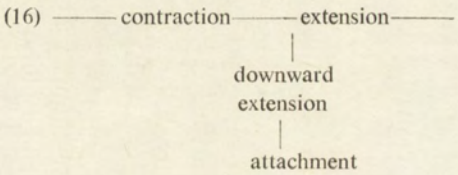
Sometimes the attachment is temporarily effected in an unusual place along the axis of locomotion: at the rear, i.e., behind the contracting part of amoeba (6), at the advancing tip, i.e., in front of its extending segments (7), and exceptionally at both these sites at one time (8). In all these cases the cell cannot effectively locomote, but the posterior contraction and anterior extension are still acting, and

[3] Concept of membrane flowing freely beside the attachment site, is hardly acceptable for the membrane and still much less for the cortical frame, because such attachment site could not constitute the point of support of any force, and it would be an attachment completely ineffective for cell locomotion.

Table 1

Principal types of co-axial and non-axial cell frame movements and their basic mechanisms

Linear distribution of contraction, extension and attachment	Other requirements	Type of movement effected
(1) —contraction——attachment——extension—	none	co-axial forward motion of whole celle frame
(2) —contraction——attachment——extension—	horizontal asymmetry of contraction	lateral bending of the posterior part of trunk and lateral bending of contracting pseudopodia
(3) —contraction——attachment——extension—	vertical asymmetry of contraction	lifting up or falling down of the posterior part of trunk with the uroid
(4) —contraction——attachment——extension—	lateral restriction of availability of extended material	lateral bending of frontal growing pseudopodia
(5) —contraction——attachment——extension—	"ventral" restriction of availability of extended material	bending of frontal growing pseudopodia towards the substrate
(6) attachment——contraction——extension—	none	co-axial backward motion of whole celf frame
(7) —contraction——extension——attachment	none	co-axial backward motion of whole cell frame
(8) attachment——contraction——extension——attachment	none	co-axial backward motion of whole cell frame
(9) attachment——contraction——extension——attachment	extra material flow from other directions	bending of whole cell in any plane
(10) —contraction—attachment—extension—attachment	none	bending of frontal pseudopodia in any plane
(11) —contraction——attachment——extension—	none	turning of whole cell by lateral pulling
lateral contraction		
attachment		

(12) 	none	side movement of whole cell by lateral pulling
(13) 	none	turning of whole cell by lateral pushing
(14) 	none	side movement of whole cell by lateral pushing
(15) 	none	lifting up and pushing back of frontal growing pseudopodia
(16) 	none	lifting up of whole cell effected from its frontal side

the whole semi-rigid cell frame performs a relative co-axial movement in the backward direction, as shown in the precedent paper.

In the case of double attachment at the front and at the rear, the cell may sometimes elongate if additional extending material is brought in from outside the principal body axis (9). Then, the whole body bends because of its excessive extension between two fixed points. More commonly two attachment points are established in line along the axis of locomotion, one at its usual place and the second in a frontal pseudopodium (10). In such a case just this growing pseudopodium undergoes bending due to the same mechanism of excessive extension.

The usual linear distribution of contraction, attachment and extension, is often counteracted by an extra attachment point established by a lateral contracting pseudopodium, which may just compete with the attachment of the principal axis of locomotion (11), or break it completely (12). In the first case, pulling force of lateral contracting pseudopodium is applied eccentrically in respect to the attachment of principal axis and the turning movement results, and in the second case only a side movement of the whole cell is produced.

Exactly the same results are induced by pushing effects of firmly attached pseudopodium extending laterally, which may eccentrically interfere with the attachment of main axis (13) and provoke turning, or overcome its resistance (14) and give rise to a side movement. Special case of the same mechanism present the anterior supporting knobs starting to extend downwards against the substrate, which wind up the principal leading pseudopodia (15), or the whole advancing front of amoeba (16), depending on the stability of other attachment sites along the principal axis of locomotion.

In the classification presented above only basic types of cell frame motion were considered, with omission of different cases of composite movements which represent just a combination of elementary situations shown in the Table 1, and add nothing to the understanding of mechanism of locomotion. Some situations imaginable but little probable, and never observed, were also left out of consideration. This is for instance the case of linear arrangement of two attachment sites with a contracting region in between, because this would generate tension breaking one of the attachments, or maintain a dynamic equilibrium and make two attachment points to behave as a single large attachment zone. In the Table 1 the possibility of torsion type of movement has been also omitted, although Rinaldi (1963) described a case of torsion of frontal pseudopodium. However, the present author never saw such a movement in amoeba, and the oblique striation present on the time-exposure picture given by Rinaldi is exactly similar to that produced

by bending pseudopodia. In fact, torsion would imply some rotation of pseudopodium around its axis and would be recorded as two oblique striations intersecting each other. But after all, this controversy may be only a question of wording and not of interpretation.

The fact that the behaviour of cell contours in normal co-axial forward locomotion, as well as their so various non-axial movements, are easily explained by the only factors of contraction-extension gradient of the semi-rigid cell frame and of differentiated attachment to the substrate, makes necessary to reconsider in some extent the general concept of locomotion of amoeba.

Implications to the General Concept of Locomotion

Explanation of all the co-axial and non-axial movements of the cell contours, as presented in this study, is based on the concept of posterior contraction and anterior expansion, which is the fundamental part of the classical theory of Mast (1926), and of the views of its more modern adherers as Goldacre and Lorch (1950), Jahn (1964), Rinaldi et al. (1975).

One can object that many facts, and even some observations described in this study, prove that contraction may occur in any body part of amoeba. This is evidently true, and in this respect the author adheres to the view expressed by Marsland (1964). One should realize however, that the posterior contraction concept applies to amoeba not exposed to local stimulation, which moves "spontaneously" forward, what means that it maintains its motory polarization by an unknown self-regulatory mechanism (the self-regulation concept proposed by Goldacre 1956 seems now obsolete). Amoeba may contract in other regions when locally stimulated, but this leads just to reorientation of its locomotory axis, as well in respect to the cell body itself as in respect to its environment, and the new axis is established again along the new gradient of posterior contraction and anterior expansion.

The posterior contraction theory allows to explain the co-axial and non-axial movements of whole cells when supplemented by its most recent developments, in particular: (1) localization of the contractile activity in the thin cortical layer of cytoplasm beneath the cell membrane (Korohoda and Stockem 1975), and not in the thick granular ectoplasm participating in the plasmasol-plasmagel conversion cycle, (2) demonstration that amoebae after removal of most part of their cytoplasm may still locomote but without streaming of endoplasm through ectoplasm cylinder (Kalisz and Korohoda 1976), (3) quantitative definition of contracting and expanding regions and

sub-regions of amoeba body and identification of contraction-expansion gradient along the main axis of locomotion (Grębecka and Grębecki 1975), and (4) analysis of position of the attachment sites in respect to the contracting and extending areas, and of its locomotory effects (Grębecki 1976).

The co-axial and non-axial movements of the whole cell contour cannot be explained by the plasmagel-plasmasol interconversion and by the endoplasm streaming, because the directions of both types of motion are in great extent independent from one another. More so, one can dispense with referring to the intracellular transport of material, because the corroboration of cortical contraction, cortical extension, and attachment to the substrate, offers by itself a satisfactory explanation of cell locomotion.

To produce movement the cell needs a structure generating the force, a point of support, and a structure rigid enough to transmit tension between the point of application of force and the point of support. The cortical cytoplasm contracts and relaxes, i.e., it consists the structure generating the force, by intermediary of the cell membrane covering it, it adheres to the ground successively at different spots and provides migrating points of support for the force action, and finally it is enough rigid to transmit through its own structure the tension created between the points of force application and the points of support.

In other words, the cell periphery of amoeba constitutes a structure comparable in its motory functions as well to the muscle as to the skeleton of higher animal. Its "skeletal" role is expressed in that it maintains the cell shape relatively more stable than the cell position in respect to the substrate (Grębecka and Grębecki 1975). The cortical cytoplasm envelopes the whole cell and for that reason it has been called by Grębecki (1976) the semi-rigid cell frame, term which has a precise physical meaning, corresponds exactly to its mechano-dynamical properties and functions, and refers clearly to the cell shape and macromorphology investigated by the author, instead of evoking micromorphological intracellular differentiation.

Since the semi-rigid cell frame generates the force, establishes the points of support, and transimts the resulting tensions, it is physically capable of autonomous movement and may be responsible alone for cell locomotion. In fact, two basic phenomena constitute the background of locomotion in amoeba: pulling the semi-rigid cell frame towards the attachment site if the attached region undergoes contraction, and pushing the cell frame out from the attachment site if the attached region is extending. It has been exposed in details in precedent chapter how

these two phenomena explain the normal co-axial forward locomotion (or the co-axial backward motion of the cell frame of non-locomoting specimens), as well as all the diversity of non-axial movements. Asymmetry of contraction or extension was only subsidiary hypothesis needed to explain some kinds of bending.

In order to maintain locomotion during illimited time, instead of performing just separate movements, every piece of cortical material should periodically change its relative position in respect to the axis of locomotion: it should enter the posterior shortening part of the cell frame when contracting, and be included into its anterior extending portion when relaxing.

One can imagin three possible modes to meet this requirement. First one would consist in a full predominance of the contraction–relaxation cycle over the motory cell polarization, i.e., the cell should automatically form the withdrawed tail in that region where the material is apt to contract, and the advancing front where it is ready to relax. However, such a mechanism would neglect the regulation of contraction–relaxation cycle, and would probably result in oscillatory movement instead of progressive locomotion.

Second possible mechanism would be the intracellular transport of contractile material by the endoplasm stream, from the contracting rear up to the extending front. This would be the same mechanism as postulated already by Mast (1926) for the granular ectoplasm, and by Goldacre (1961) for the cell membrane, but when applied to the contractile cortex it would obviously need a periodical disintegration of the actine filaments and their reconstitution and reattachment to the cell membrane.

Third mechanism, which seems most plausible to the author, is linked with the folding and unfolding of the cell membrane, as demonstrated by Czarska and Grębecki (1966) and further studied by Haberey et al. (1969) and Stockem et al. (1969). The folding-unfolding process accounts for the transport of the cell membrane over the surface of moving cell instead through its interior, and now it seems conceivable that the membrane folds, unfolds, and shifts forwards, together with the contractile layer lining its interior face. This concept offers at the same time a mode of cortex cycling *in situ* and a driving force for membrane folding. Only condition needed for such a system to operate, would be absence of sliding inside the cortex between the membrane and the contractile layer, but instead a possibility of sliding between the cortex and the granular ectoplasm. This postulate needs experimental testing in future.

Implications of the concept of locomotion presented above, for the interpretation of behaviour of granular cytoplasm in the cell interior, may be exposed in the following way:

All the granular cytoplasm behaves probably in a rather passive manner in small amoeboid cells, and in *A. proteus* with removed endoplasm (Kalisz and Korohoda 1976). It is simply carried forwards by the advancing cortical frame and this forcible movement is limited to the primitive form of bulk transport. In normal large amoebae the intracellular movement assumes much more complicated pattern of the ectoplasm-endoplasm interconversion with the axial endoplasm stream. Nevertheless, even in this case, the movement might be considered to be passive, if its motive force is looked for not inside the circulating material, neither in the endoplasm core (Allen 1961) nor in the ectoplasm cylinder (Mast 1926), but outside the whole system: in the contraction of posterior regions of the cortical layer which does not take part in the intracellular ectoplasm-endoplasm conversion cycle. On the other hand however, the recent advances in study of motile properties of the naked cytoplasm extracted from large amoebae allow to presume that the circulation of the granular layers may be propelled by the forces generated inside them. If so, the cortex would co-operate in producing streaming phenomena, probably by providing the points of attachment for the contractile fibrils, or in other words, the points of support for the contraction forces generated in the ectoplasm and/or in the endoplasm.

In any way, the intracellular transport of cytoplasm is not considered here as primary factor of cell locomotion, as in the classical tail contraction theory and in the frontal contraction hypothesis. As far as the mechano-dynamical aspects are considered alone, there is no causal relation between the intracellular streaming and locomotion, but they are two independent movements driven by the same common force: contraction of the cortex, or by two separate forces.

Mechanically, the endoplasm streaming brings only a secondary contribution to cell locomotion by playing a subsidiary role in transmission of tension along the main body axis: the outflow of endoplasm from the contracting regions corroborates their collapse and its inflow into the extendings regions corroborates their expansion.

On the contrary, the endoplasm streaming certainly plays a major indirect role in locomotion of large amoebae, what results from its principal function of circulating the metabolites in the cell interior, as recently stressed by Kalisz and Korohoda (1976). First, it may carry organic poliphosphates towards the sites of contraction. Secondly, it probably redistributes substances which regulate the contraction–

relaxation cycle (Grębecka 1977), and in this way it maintains the cell polarization necessary for effective locomotion.

The present concept of amoeboid movement has many common points with the Mast-Goldacre-Jahn-Rinaldi line of thinking, and it seems therefore useful to stress in conclusion its basic analogies and major differences in respect to the classical theory of posterior contraction.

The essential analogy consists in that the contraction is postulated to take place in the posterior part of the cell, and that it is supposed to press out the endoplasm forwards. The following differences seem most important: (1) the contraction force effective in locomotion is generated in the thin outer layer of cortical cytoplasm forming a semi-rigid cell frame, and not in the thick cylinder of granular ectoplasm, (2) tensions which produce movement are transmitted by the semi-rigid cell frame between the points of force application and the points of substrate attachment, and not by the cell interior from the contracting tail to the advancing front, (3) cell locomotion and intracellular streaming are thought to be mechanically independent phenomena which have just a common origin, instead of considering the intracellular streaming as primary causal factor of locomotion, (4) the contractile structures may probably keep their integrity during locomotion and change their relative position by sliding over the cell periphery, instead of always being periodically disintegrated and transported through the cell interior.

RÉSUMÉ

Les plus importantes catégories des mouvements qui s'effectuent dans des directions différentes de celle de l'axe locomoteur principal, peuvent être définies de la manière suivante: les mouvements latéraux consistent en ce que l'axe de la cellule est repoussé du côté sur toute sa longueur, les mouvements de détour se manifestent quand l'axe de locomotion est réorienté et la cellule pivote sur place comme un seul bloc, et enfin les mouvements de fléxion qui concernent soit le tronc de l'amibe soit les pseudopodes seuls. Les mouvements de ce genre peuvent se produire aussi bien sur le plan horizontal que dans le sens vertical. Toute la variété de mouvements percevables comme déplacement du contour extérieur de l'amibe, soit dans le sens de l'axe principal soit dans d'autres directions, c'est-à-dire en réalité toute la locomotion, peut être expliquée par: le gradient postério-antérieur de la contraction et l'extension de la structure semi-rigide encadrant la cellule sous forme de cortex, le déplacement progressif des sites d'attache de la cellule à la surface qui constituent les points d'appui pour l'action des forces de la contraction et l'extension, et par la transmission des tensions par le cortex lui-même. Le courant cytoplasmique intracéllulaire est considéré comme un phénomène dans une grande mésure indépendant, et non comme le premier moteur de la locomotion.

REFERENCES

Allen R. D. 1961: A new theory of ameboid movement and protoplasmic streaming. Expl. Cell Res., (Suppl.), 8, 17–31.

Bell L. G. E. and Jeon K. W. 1963: Locomotion of *Amoeba proteus*. Nature, 198, 675–676.

Bovee E. C. and Jahn T. L. 1973: Locomotion and behaviour. In: The Biology of *Amoeba*, Academic Press, 250–290.

Czarska L. and Grębecki A. 1965: Rotary movement in *Amoeba proteus*. In: Progress in Protozoology, Abstr. Second int. Conf. Protozool. London, 1965, Abstr. 327.

Czarska L. and Grębecki A. 1966:: Membrane folding and plasma-membrane ratio in the movement and shape transformation in *Amoeba proteus*. Acta Protozool., 4, 201–239.

Goldacre R. J. 1956: The regulation of movement and polar organisation in *Amoeba* by intracellular feedback. Proc. Ist Internat. Congr. Cybernetics, 715–725.

Goldacre R. J. 1961: The role of the cell membrane in the locomotion of amoebae, and the source of the motive force and its control by feedback. Expl. Cell Res., (Suppl.), 8, 1–16.

Goldacre R. J. and Lorch I. J. 1950: Folding and unfolding of protein molecules in relation to cytoplasmic streaming, amoeboid movement and osmotic work. Nature, 166, 487–499.

Grębecka L. 1977: Behaviour of anucleate anterior and posterior fragments of *Amoeba proteus*. Acta Protozool., 16, 87–105.

Grębecka L. and Grębecki A. 1975: Morphometric study of moving *Amoeba proteus*. Acta Protozool., 14, 337–361.

Grębecki A. 1976: Co-axial motion of the semi-rigid cell frame in *Amoeba proteus*. Acta Protozool., 15, 221–248.

Haberey M. 1971: Bewegungsverhalten und Untergrundkontakt von *Amoeba proteus*. Mikroskopie, 27, 226–234.

Haberey M., Wohlfarth-Bottermann K. E. und Stockem W. 1969: Pinocytose und Bewegung von Amöben. VI. Mitteilung. Kinematographische Untersuchungen über das Bewegungsverhalten der Zelloberfläche von *Amoeba proteus*. Cytobiologie, 1, 70–84.

Jahn T. L. 1964: Relative motion in *Amoeba proteus*. In: Primitive Motile Systems in Cell Biology, Academic Press, 279–302.

Kalisz B. and Korohoda W. 1976: Experimental study on locomotion of *Amoeba proteus*. I. Movements in the nucleated and anucleated fragments of the amoebae after removal of the part of their cytoplasm. Acta Protozool., 15, 345–358.

Korohoda W. and Stockem W. 1975: On the nature of hyaline zones in the cytoplasm of *Amoeba proteus*. Microsc. Acta, 77, 129–141.

Marsland D. 1964: Broad concept of the tube-wall contraction hypothesis. In: Primitive Motile Systems in Cell Biology, Academic Press, 331–332.

Mast S. O. 1926: Structure, movement, locomotion and stimulation in *Amoeba*. J. Morph., 41, 347–425.

Rinaldi R. A. 1963: Velocity profile pictographs of amoeboid movement. Cytologia, 29, 417–427.

Rinaldi R. A., Opas M. and Hrebenda B. 1975: Contractility of glycerinated *Amoeba proteus* and *Chaos chaos*. J. Protozool., 22, 286–292.

Stockem W., Haberey M. und Wohlfarth-Bottermann K. E. 1969: Pinocytose und Bewegung von Amöben. V Mitteilung. Konturveränderungen und Faltungsgrad der Zelloberfläche von *Amoeba proteus*. Cytobiologie, 1, 37–57.

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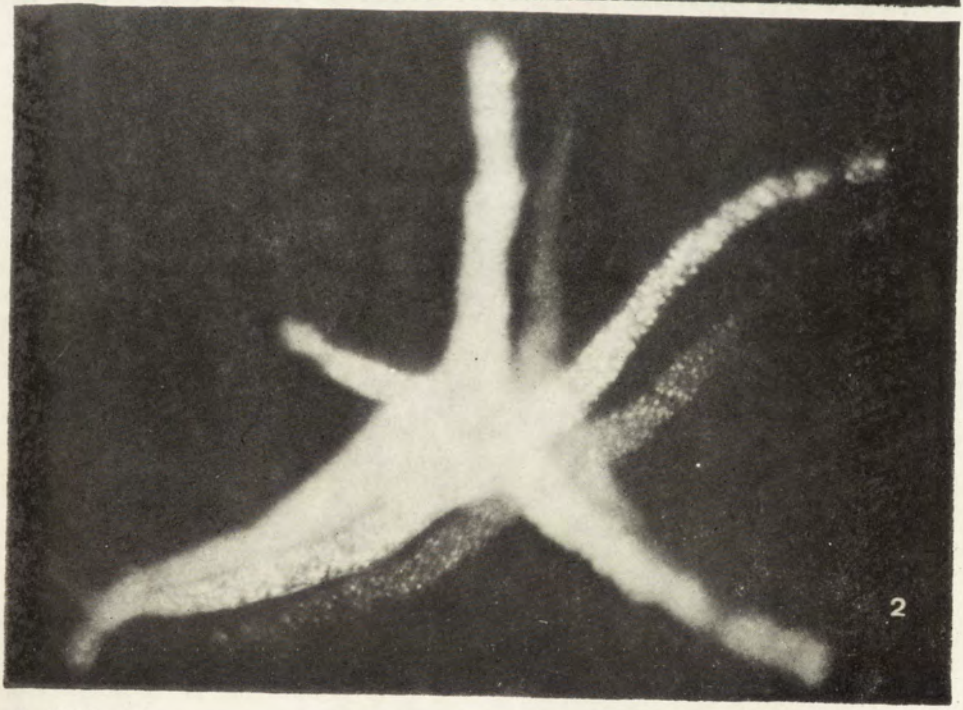
EXPLANATION OF PLATES I–VI

1–2: Side movements of whole cells, as recorded by the time-exposure and the double-exposure techniques
3: Turning movement of whole amoeba (time-exposure)
4–5: Bending the anterior body part in two polypodial amoebae, shown by the time-exposure and by the double-exposure photography
6–7: Facultatively monopodial amoebae, one manifesting bending movement in its frontal part, and another one bending in its posterior region (time-exposure)
8–9: Bending movements of lateral pseudopodia manifested either in the distal part, either in the proximal segment (time-exposure)
10–13: Short-duration movements of pseudopodia, as recorded by time-exposure, with the initial and final contours seen as well as the non-axial striation
14: Time-exposure picture of pseudopodium which was bending in opposite directions in its proximal and its distal segments
15–16: Examples of non-axial movements manifested in parallel by two neighbour pseudopodia (time-exposure)
17–21: Double-exposure records of bending movements of advancing pseudopodia

All the time-exposure pictures were taken with 20 s recording time, and all the double-exposure ones with 20 s interval between both exposures (brighter image corresponds always to the second exposure)



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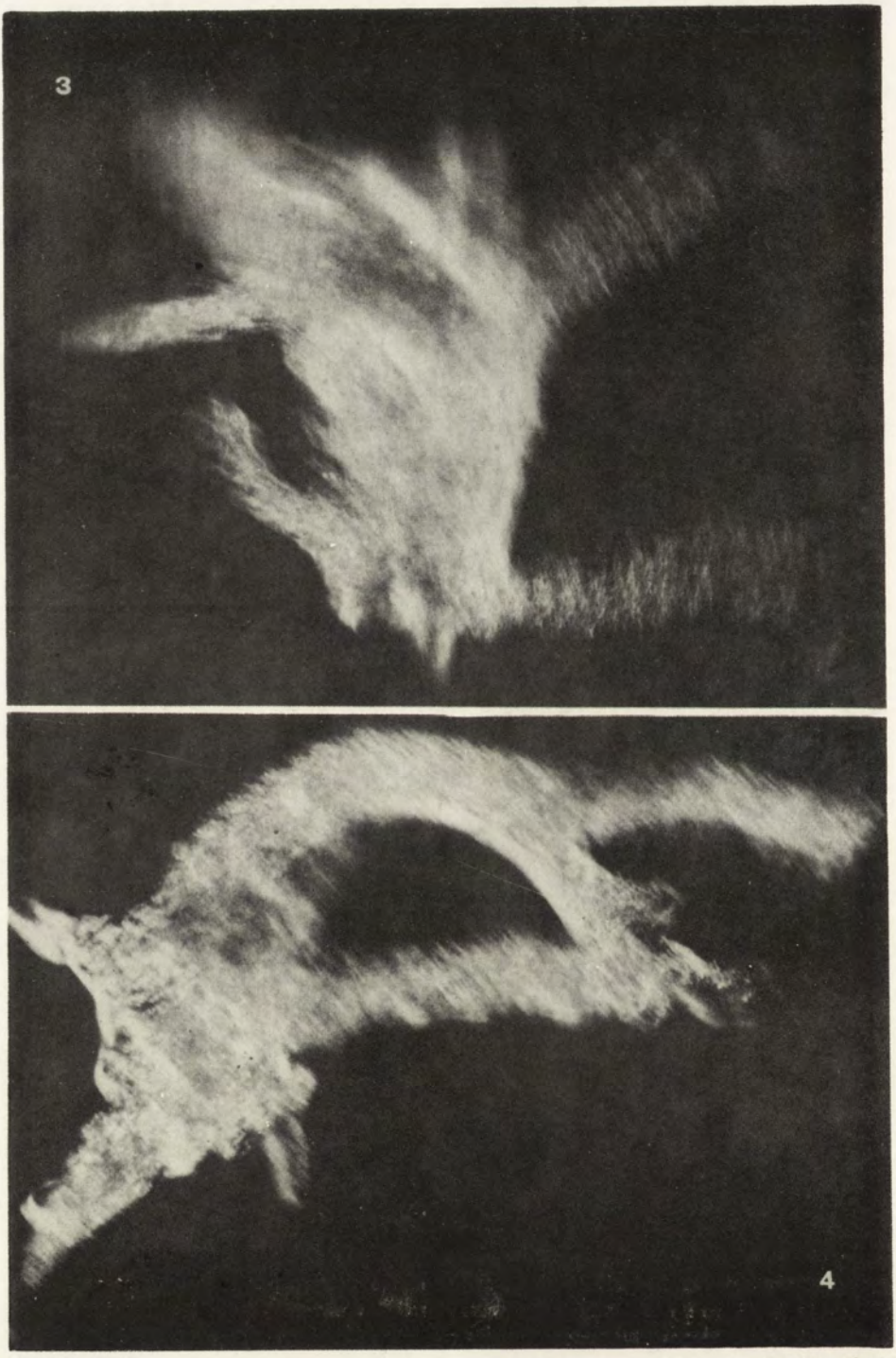

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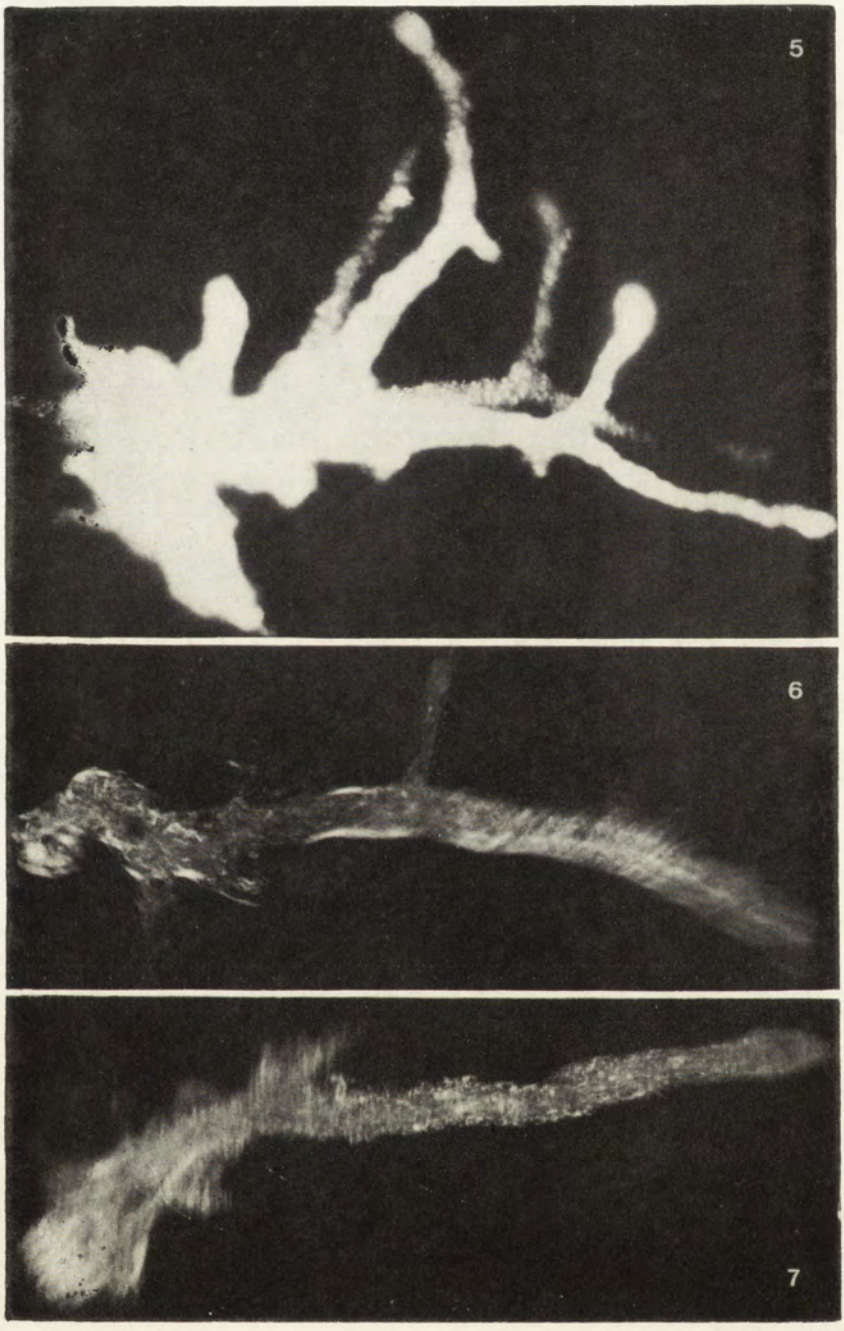

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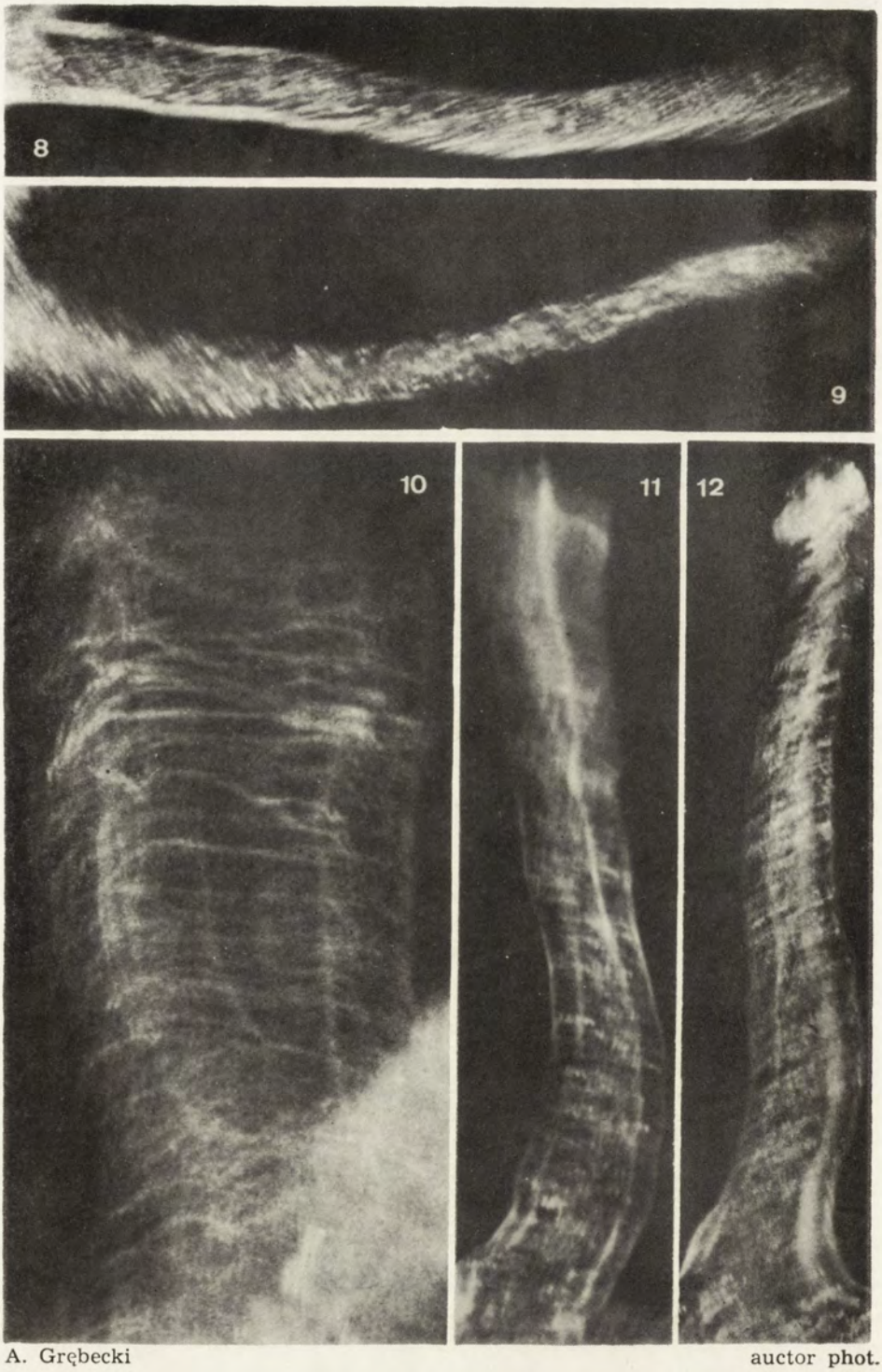

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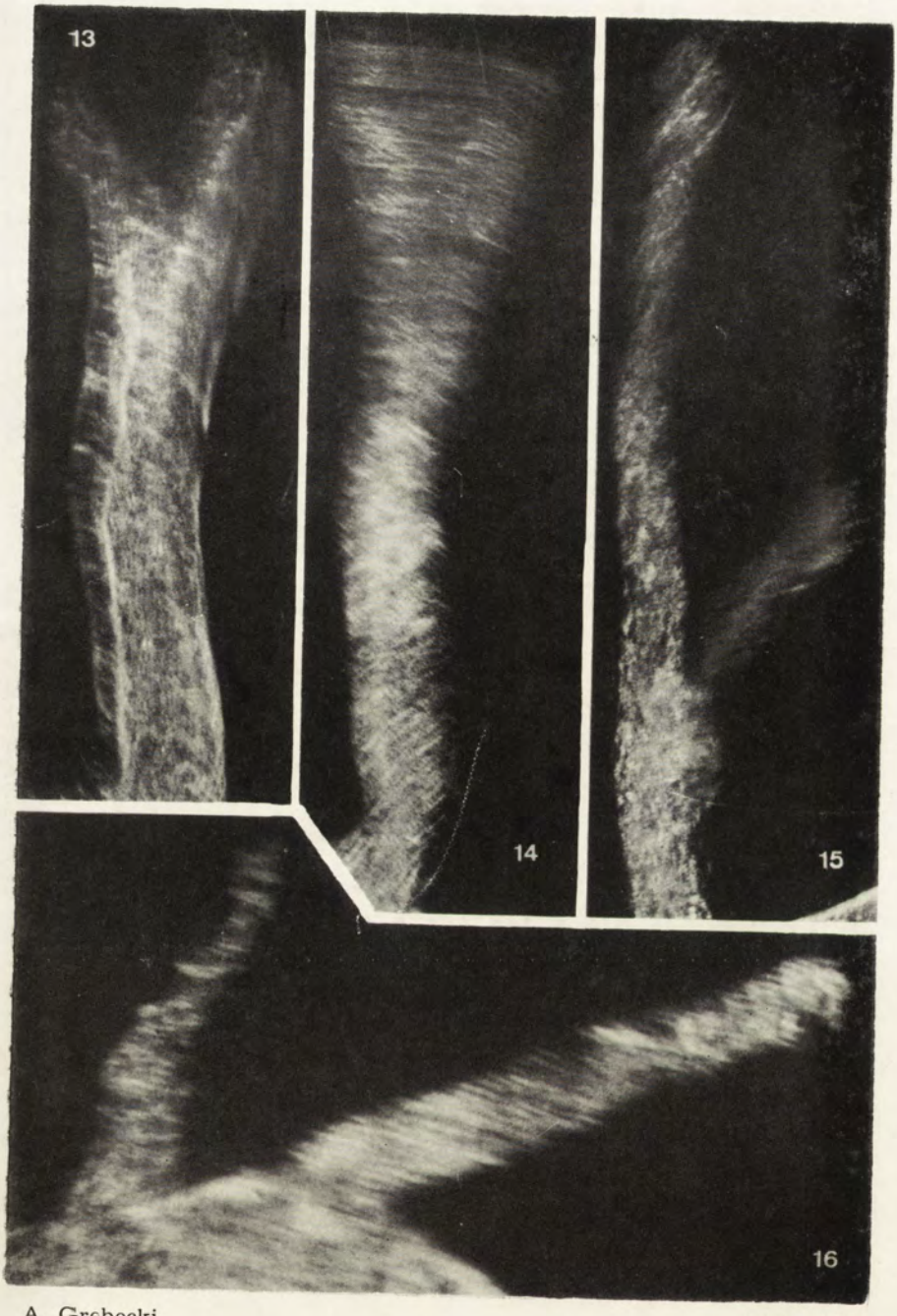

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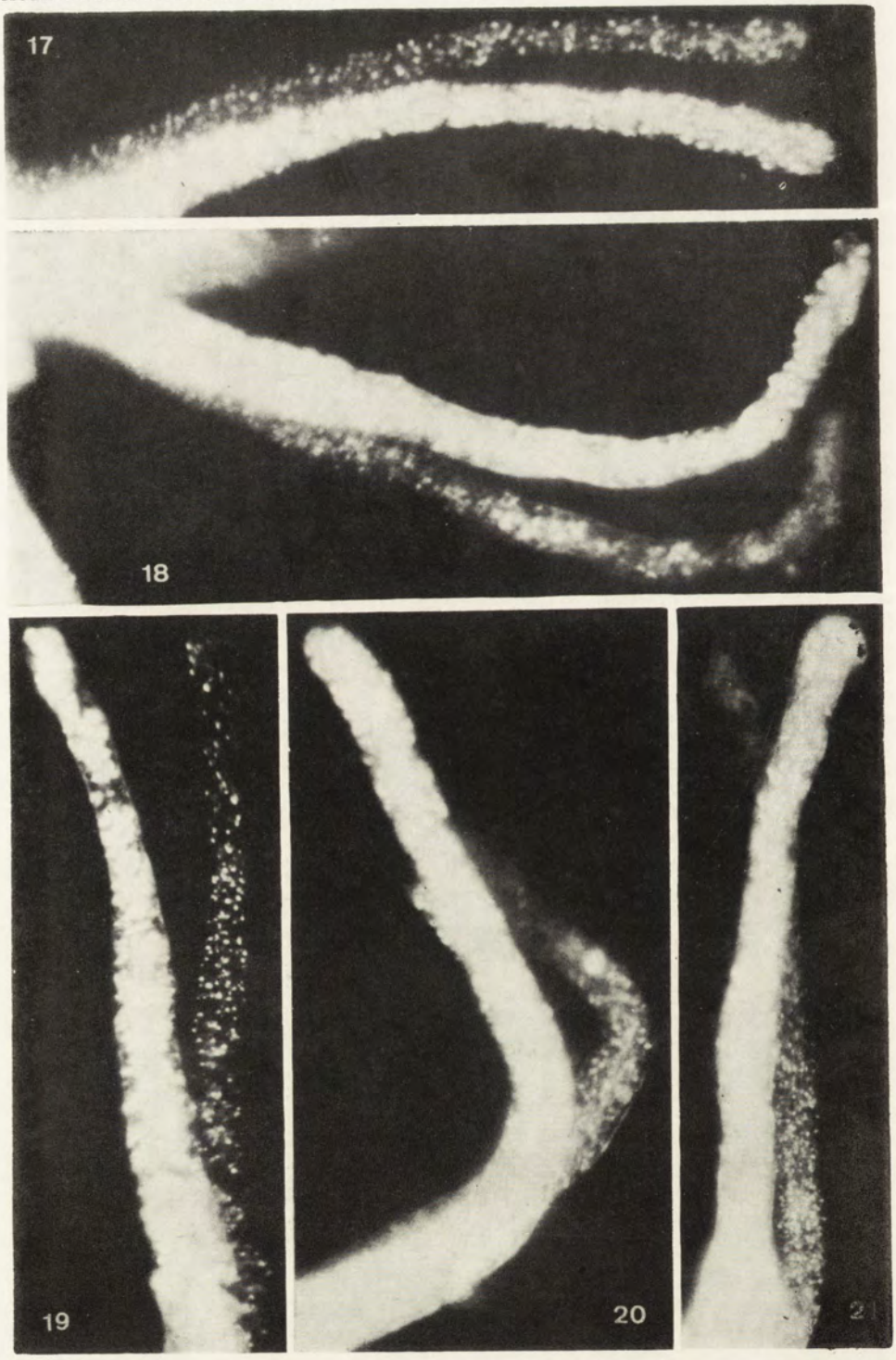

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Behaviour of Anucleate Anterior and Posterior Fragments of *Amoeba proteus*

Synopsis. Anucleate fragments obtained by dissection effected just in front of the nucleus keep their motility sensibly longer than those cut off just behind the nucleus. Moreover, the anterior anucleate fragments show their surface smooth, and the posterior ones immediately corrugate. Spontaneous or artificial inversion of cytoplasmic stream before bisection, makes the posterior fragments to behave like anterior ones. Motility and surface conformation of a fragment is not depending on its situation vs. the external morphology but on its former upstream or downstream position in the cytoplasmic flow, in respect to the nucleus. This result speaks against the "tail organizer" theory of Goldacre and Lorch, but it allows to suggest that the unstable proteins bidirectionally migrating between the nucleus and the cytoplasm, as discovered by Goldstein and co-workers, play the role of regulatory factor of motility by executing relaxatory function, and that their distribution in the cell interior is effected by the cytoplasmic stream.

Probably Gruber (1886) was the first to cut amoebae in two parts and to note the relation between the presence of the nucleus and locomotion, although the recent authors commonly assign the priority to Hofer (1890). The significance of nucleus for locomotion of amoebae was denied only by Stolć (1910), but confirmed by Willis (1916), Lynch (1919) and Becker (1926). All these early authors are quoted here after Becker (1926). More recent observations, not only of anucleate fragments but also of enucleated and renucleated, mitotic and intermitotic specimens, confirmed without any doubt the significant fact that the nucleus is necessary as well for the coordinated movement as for the cell attachment to the substrate (Clark 1942, 1943 and 1944, Lorch and Danielli 1950 and 1953, Goldacre and Lorch 1950, Brachet 1955, Goldstein and Jelinek 1966, Jeon 1968, Ord 1968, Lorch 1969 a and b, Kalisz and Korohoda 1976). At the same time, first concepts of the possible mechanisms of the nuclear influence upon locomotion, are put forwards by some authors.

Goldacre and Lorch (1950) postulate that the interphase nucleus produces two "tail organizers" which are released during mitosis into the tail regions of daughter cells. They contain an enzyme needed to produce ATP or an ATP-like substance from the precursor present in the cytoplasm. During the cell life the "tail organizer" becomes exhausted, and then amoeba ceases movement and rounds up, prior to cell division. This explanation agrees with the tail contraction theory of amoeboid movement in the presentation of Goldacre and Lorch (1950) and Goldacre (1961).

Goldstein and Jelinek (1966) found that anucleate fragments cut from mitotic cells continue to show normal amoeboid movement for a several hours. Similar results were obtained by Lorch (1969 a, b and 1973) who cut anucleate fragments from early postdivision cells. On the basis of these experiments suggestion has been made by Goldstein (1973) that some substances of nuclear origin (probably proteins) are involved in the mechanism of amoeboid movement, and that the normal movement of interphase cells is probably depending on a continuous supply of these proteins from the nucleus to the cytoplasm.

In the present work two kinds of anucleate fragments were observed: anterior fragments cut just in front of the nucleus, and posterior fragments cut just behind the nucleus. The difference between the anterior and posterior anucleate fragments from the standpoint of the "tail organizer" theory is self-evident. On the other hand, if a substance emanating from the nucleus and involved in the movement of amoeba, was continuously supplied into the cytoplasm, its concentration should be higher in the anterior part of amoeba than in its posterior part because of the direction of cytoplasmic streaming which is flowing around the nucleus. The substance in question, after leaving the nucleus, should be carried by the stream forward, to the advancing front of amoeba. So, one could expect a difference in duration and/or in character of movement, just after cutting, between the anterior and the posterior anucleate fragments.

Material and Methods

Material used in this study was *Amoeba proteus* (interphase cells) cultured in Pringsheim medium, and fed with *Colpidium* grown in yolk cultures.

De Fonbrune micromanipulator was used in all experiments. Tools for bisections and microinjections were made in the De Fonbrune microforge. Amoebae were put for operation into an oil chamber, and after that they were kept in the same chamber for further observation and taking pictures.

The following precautions were taken in all the cases: (1) the interphase speci-

mens only were taken for experiments, (2) individuals sensibly larger or smaller than the average size were rejected, (3) fragments similar in size were selected for conclusive observations, (4) the pattern of cytoplasm streaming was carefully controlled and noted before each bisection, (5) the anucleate fragments were observed for at least 30 min after operation.

Nevertheless, the data obtained are presented just in form of most typical and most frequent results, which may serve to estimate differences in the behaviour of various kinds of fragments, but intentionally they were not treated in any statistical manner. The reason is that, in spite of all precautions enumerated above, the fragments are not exactly uniform, and moreover, the pattern of intracellular streaming in amoebae before the operation, which is individualized in very high degree, may greatly influence the further fate of fragments. As far as the duration of fragments motility is concerned, extreme values for each group (7–20 cases) are given on the drawings, and on the photographic pictures the individual values are indicated, these which concern the specimen recorded.

Results

Anucleate Fragments Cut from Polypodial Amoebae

In the first series of experiments amoebae, polypodial in shape and manifesting the usual pattern of locomotion, were cut just in front of the nucleus. The nucleate posterior parts continued normal movement during illimited time. However, the anucleate anterior fragments also moved normally (just slightly slower) for periods going from 9 to 20 min. Only after that time their movement gradually ceased and the fragments rounded up. It is very characteristic that at this stage their surface is smooth, and its corrugation appears much later. The Pl. I shows the example of a specimen dissected in this manner, after what the posterior part containing the nucleus moves normally all over the sequence, and the anterior anucleate one maintains locomotion at the first stages and after reaching the rounding up phase it keeps its surface smooth.

In the second series of operations, the normal moving specimens were dissected just behind the nucleus, in order to cut out an anucleate posterior fragment. As in the former case, the nucleate part kept indefinitely the regular pattern of locomotion. The anucleate posterior fragments move only 15 s up to 5 min 30 s in extreme cases, then they stop, round up and become immediately corrugated. Such picture is shown by the Pl. II, which brings as example a sequence of events recorded during the first and the second minute after dissection.

Generalized schemes of behaviour of the anucleate fragments, as dependent on their position in respect to the nucleus and to the main body axis, prior to the operation, is presented in the Figs. 1 and 2.

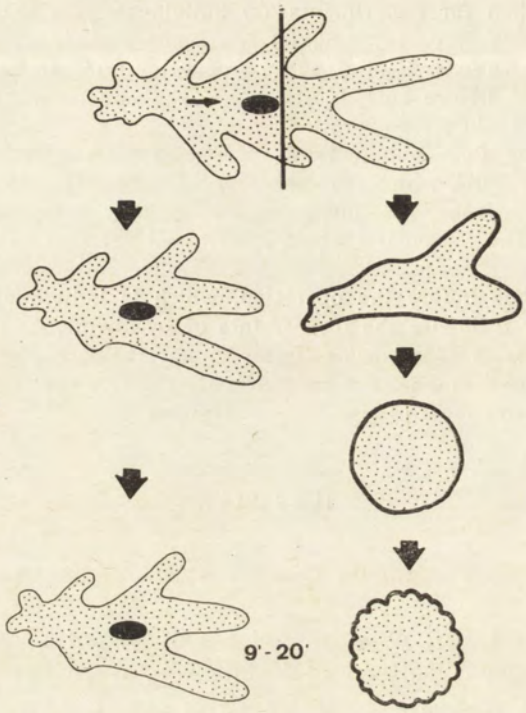

Fig. 1. Results of bisection effected in polypodial amoeba just in front of the nucleus: the anterior anucleate fragment initially preserves its motory capacity, and after rounding up it passes through the stage of a smooth sphere

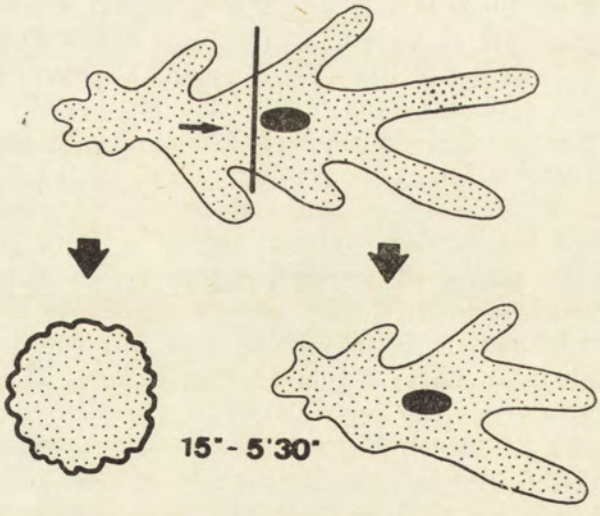

Fig. 2. Results of bisection effected in polypodial amoeba just behind the nucleus: the posterior anucleate fragment almost immediately looses mobility, and instantly assumes the form of a corrugated sphere

Different time of maintaining the motility, and different surface conformation after the cessation of movement, are the main characters which clearly distinguish the anterior and the posterior anucleate fragments between them.

In the third series of experiments, the posterior part of amoeba was sectioned twice behind the nucleus. First operation consisted in resection of the "original tail", and the second one (effected in the second minute of experiment) in cutting off the "secondary tail" which is arising in the zone of first bisection. As shown in the Fig. 3 and in

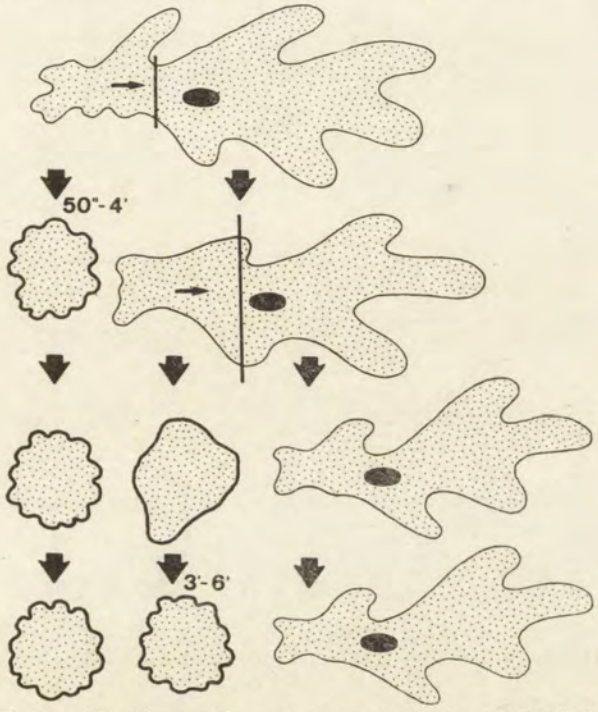

Fig. 3. Results of two bisections effected successively in polypodial amoeba, across the posterior regions behind the nucleus: the first one, more posterior, anucleate fragment does not move and corrugates immediately, whereas the second one, closer to the nucleus, shows initially some motory activity and passes through the stage of a smooth clod, before it corrugates

the Pl. III, both posterior anucleate fragments, cut from the same specimen, differ in their motility and surface conformation. The "secondary tail" needs more time to cease its locomotion and goes through the smooth surface phase, before it corrugates. Its behaviour may be placed someway in between the usual standards of behaviour of the posterior and the anterior anucleate fragments, what is probably related to the fact that its contact with the nuclear influence was more recent than that of the "original tail".

Anucleate Fragments Cut from Monopodial Amoebae

The monopodial amoebae, spontaneously appearing in cultures, have extremely stable polarization, and the cytoplasmic streaming is permanently directed towards the so-called hyaline cap located at the unique advancing front. The results of bisection of such specimens, as presented in the Figs. 4 and 5, are essentially similar to those described above for polypodial cells. The anterior anucleate fragments move sensibly longer and eventually transform into smooth spheres, whereas the posterior anucleate fragments stop locomotion and corrugate almost

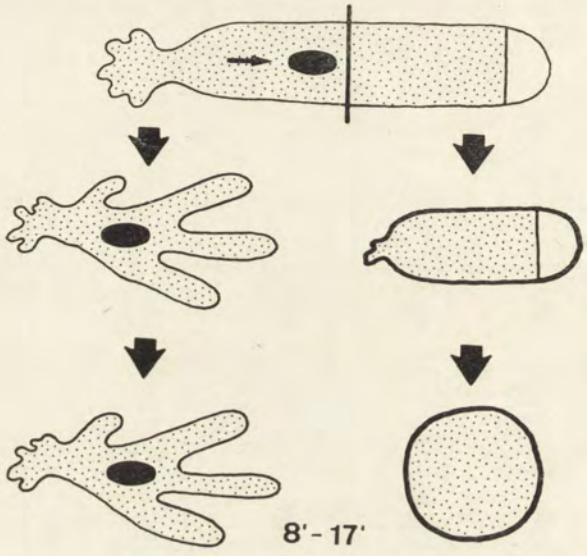

Fig. 4. Results of bisection of native monopodial amoeba just in front of the nucleus: the anterior anucleate fragment preserves motility before rounding up and even thereafter it still keeps its surface smooth

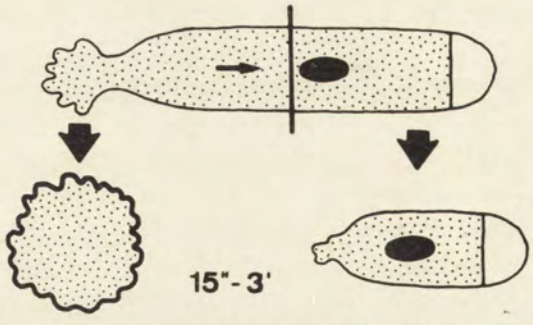

Fig. 5. Results of bisection of native monopodial amoeba just behind the nucleus: the posterior anucleate fragment almost immediately stops and corrugates

immediately. Significant particularities to be noted in addition are that, the posterior halves never reconstitute frontal caps even if they contain the nucleus, and that the anterior anucleate fragments preserve their typical fountain-like streaming pattern as long as they are still capable to move.

Effect of Spontaneous Reversals of the Cytoplasmic Stream

Further bisections were performed on polypodial amoebae which assume temporarily a V-like shape, being essentially composed of the uroid and two long pseudopodia, one of them containing the nucleus. For a certain time the directions of cytoplasm streaming may oscillate in both pseudopodia, until one of them becomes eventually the leading one and the second definitely contracts. This status of amoeba and the result of bisection are schematically drawn in the Fig. 6.

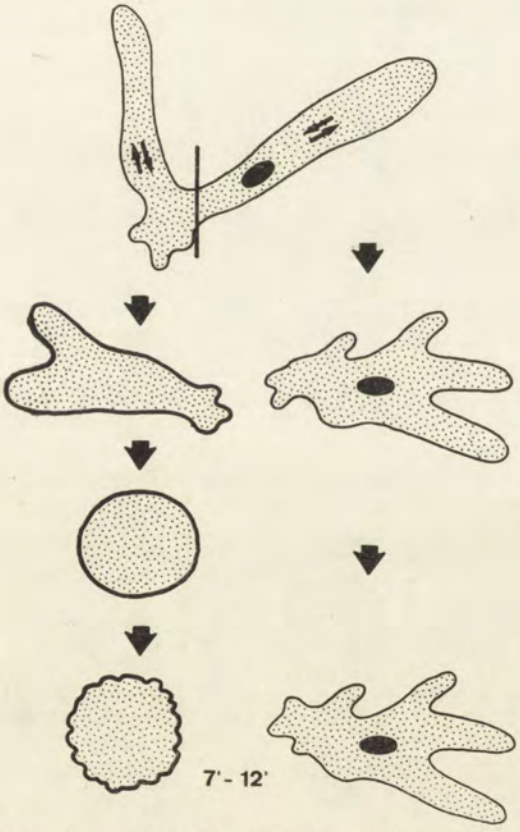

Fig. 6. Results of bisection of V-shaped polypodial amoeba with oscillations of the cytoplasm streaming direction: the anucleate fragment containing the uroid and one pseudopodium passes through the moving phase and the smooth surface stage, until it eventually corrugates

The pseudopodium containing the nucleus, after cutting it off, behaves as all nucleate fragments. The posterior half consisting of the uroid and of the pseudopodium without nucleus, behaves merely like an anterior, and not like a posterior anucleate fragment.

In normal polypodial amoebae one may sometimes observe a spontaneous definite reversal of cytoplasmic stream, which starts to flow towards the uroid. Such amoebae were bisected, as shown in the Fig. 7, at the moment when the inverse stream had already invaded the former posterior body part, but amoeba had not yet reshaped its original external morphology. The results presented in the Fig. 7 and in the

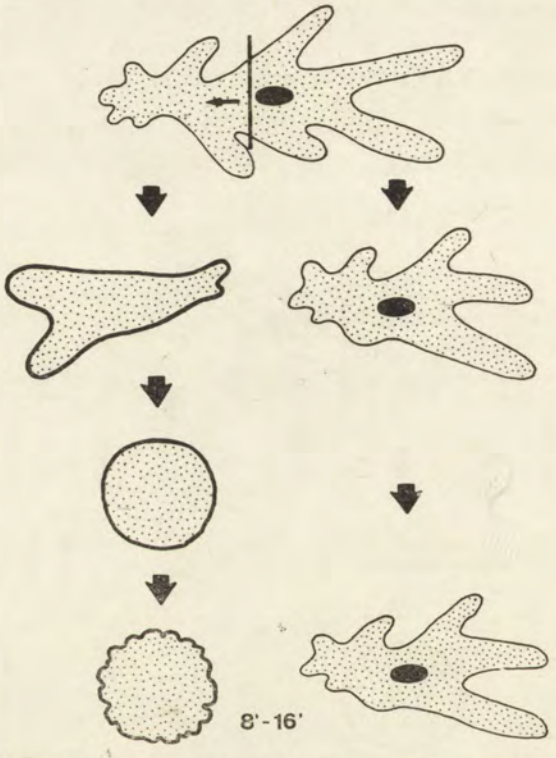

Fig. 7. Results of bisection just behind the nucleus effected in polypodial amoeba at the beginning of a spontaneous reversion of the cytoplasmic stream: the posterior anucleate fragment which has been invaded, before cutting, by the endoplasm coming from the nuclear region, keeps initially its capacity to move and preserves smooth surface even some time after rounding up

Pl. IV demonstrate that, also in this case, the reversal of cytoplasm streaming makes the fragment, morphologically posterior, to behave more like an anterior one.

The similar results obtained in both last series of experiments, in amoebae manifesting either an oscillatory cytoplasm flow, either the

initial phase of definite reversal of streaming, confirm the author's hypothesis that the differences in the motory behaviour of the anterior and posterior anucleate fragments, are related to their upstream or downstream position in respect to the nucleus, and not to other morphological features.

Effect of Artificially Induced Changes of the Cytoplasmic Stream

Dramatic changes in the pattern of streaming in amoeba are obtained after injection of a droplet of paraffin oil through the cell interior, against the inner face of the cell membrane. Vigorous streaming is immediately directed towards the injected droplet, a huge pseudopodium is formed and amoeba becomes typically monopodial, even with a net fountain, with this peculiarity only that its front is leaded by an artificial "oil cap" instead the native structure called "hyaline cap". The technique of injecting oil drops against the inner face of the membrane has been first described without ambiguity and successfully applied for *Amoeba proteus* by Goldacre (1961)[1], the further fate of artificial monopodial amoebae obtained on that way was studied by Czarska

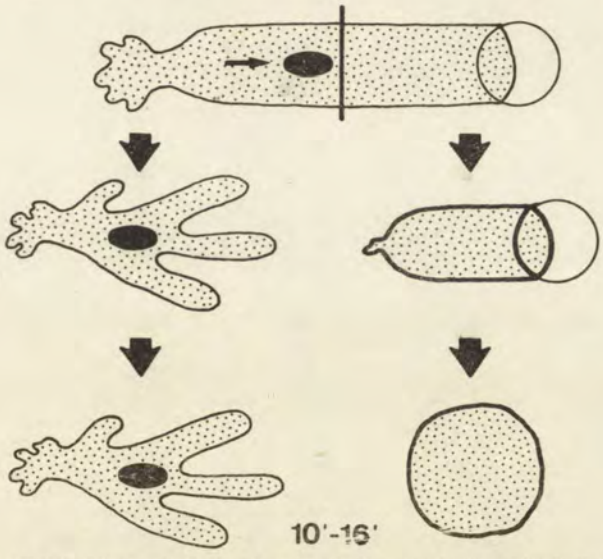

Fig. 8. Results of bisection just in front of the nucleus effected in amoeba artificially transformed into monopodial type by oil injection: the anterior anucleate fragment first maintains movement and finally becomes a smooth sphere

[1] Earlier, Dawson and Belkin (1928 and 1929) and Marsland (1933) obtained the phenomenon of "capping" in *A. dubia*, but they applied the oil from the outside, and they were unable to produce any durable effects in *A. proteus*. Marsland summarizes: "... of these species of amoeba *dubia* is the only one in which the capping reaction readily takes place".

and Grębecki (1966), and the aspects of the cell polarity changes after oil injections are recently analyzed by the present author elsewhere (Grębecka 1977). In the present study the oil injections served just as technique to provoke the cytoplasm to flow in the desired direction, before cutting out the anucleate cell fragments.

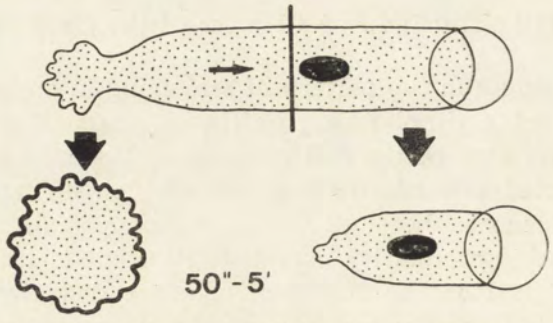

Fig. 9. Results of bisection effected just behind the nucleus of artificially monopodial amoeba obtained by oil injection: the posterior anucleate fragment does not move and quickly corrugates

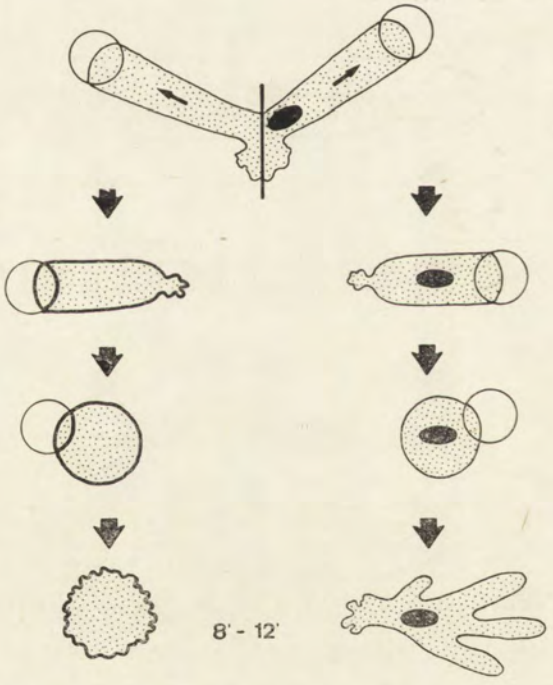

Fig. 10. Results of bisection of polypodial amoeba with the cytoplasmic stream artificially divided, by injection of two oil droplets, in two separate branches: the anucleate half moves for a certain time and even after rounding up it initially keeps its surface smooth

The behaviour of artificially monopodial amoebae after bisections effected in front of the nucleus (Fig. 8 and Pl. V), or behind it (Fig. 9), is exactly similar, as to the time of motility of anucleate anterior and posterior fragments and as to their shape evolution, to the respective results obtained with natural spontaneously monopodial specimens (cf. Figs. 4 and 5).

Double injection of two oil droplets were done to produce amoebae with endoplasm streaming separated into two opposite branches. The bisection of such a form, as shown in the Fig. 10, results in producing an anucleate half which activity seems to be situated between the behaviour of the anterior and the posterior anucleate fragments. It is eventually transformed into a corrugated sphere like posterior fragments, but that needs some 10 min to be accomplished and meantime it manifests regular movement and passes often through the phase of a smooth sphere like the anterior fragments. Results of this experiment are in large extent comparable with the effects of dissecting a polypodial V-shaped amoeba (cf. Fig. 6). An individual example of behaviour of

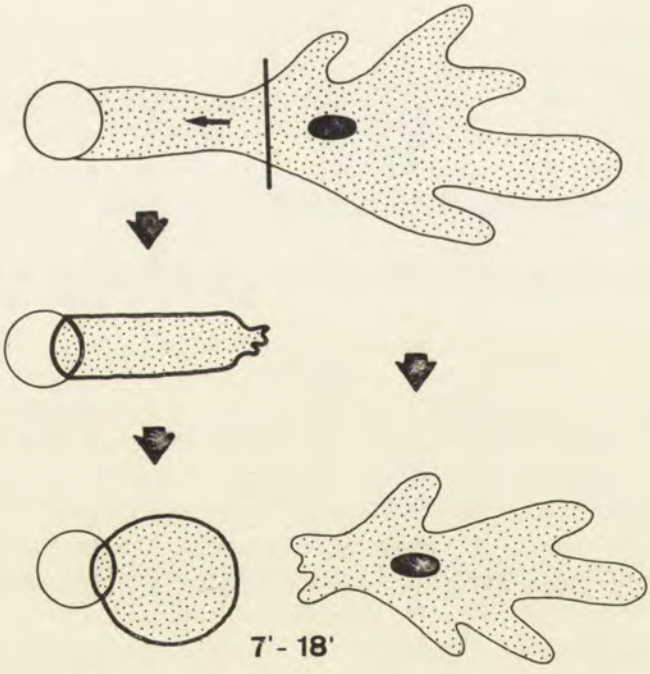

Fig. 11. Results of bisection behind the nucleus effected in polypodial amoeba at the initial phase of artificial inversion of the cytoplasm streaming induced by oil droplet injected into the uroid: the posterior anucleate fragment initially preserves its movement and keeps smooth surface after rounding up

the nucleate and anucleate halves of amoeba with two injected oil droplets, after dissection, is shown in the Pl. VI.

Injections of the paraffin oil under the uroidal membrane results in the immediate reversal of direction of the cytoplasmic stream. The posterior parts of amoebae were cut off, behind the nucleus, just after the artificial reversion of streaming has been induced. The operation and its result are shown on the Fig. 11. The effects are similar, and even more pronounced, as in the case of spontaneous reversal of cytoplasm flow direction (cf. Fig. 7), i.e., again the anucleate fragment which is posterior as far as the external morphology is concerned, behaves like anterior fragments.

In general, the artificial inversion of cytoplasm streaming produces the same effects as its spontaneous reversals: raising the motility of former posterior fragments after they are invaded by the endoplasm flowing from the nucleus side.

Discussion

Although the effects of cutting amoebae in the nucleate and the anucleate parts were described by many early authors (see the introduction), it seems impossible to refer to them in discussion because of a substantial difference of approach: the cuts were used in the present study as a means to demonstrate the different behaviour of fragments lying upstream or downstream in respect to the cell nucleus. The possible existence of such a difference, related to the former position of a fragment in respect to the streaming direction, has not been taken into account by earlier authors, and therefore, the original pattern of streaming before the operation is never defined in their reports. It should be stressed also that the present material provides no background to discuss the long-term effects of the nucleus absence, the spontaneous recovery of motility for brief periods after a few days (Clark 1942, 1943 and 1944), neither the relation between the motory efficiency and the genetic viability of re-implanted nuclei (Jeon 1968). On the contrary, it becomes necessary to discuss the concepts known in the literature which deal with the possible migration, between the nucleus and the cytoplasm, of hypothetical substances involved in producing and/or regulating the motory functions.

All the earlier experimental reports on enucleation or cutting off the anucleate fragments of *A. proteus* agree that one of the major effects of such operations is the loss of motility in specimens or fragments

without nuclei, and that it cannot be attributed to any direct operational shock. The theory of tail organizers put forward by Goldacre and Lorch (1950) is until know the only one elaborated concept of the role of cell nucleus in amoeboid movement. Basic presumption of this hypothesis is that, in normally migrating amoeba, the tail is a reservoir of the definite stock of substance needed to maintain motion, which becomes gradually dispersed in the cell interior, and is used up when reacting with the cytoplasmic ATP-precursor.

Goldacre and Lorch (1950) suggest further that the substance in question, or the products of its decomposition, migrate to the nucleus, where they are stored until the next cell division. An excessive content of this hypothetical substance, or of its decomposition products due to the intense ATP production, would lead to cell "bubbling" (= corrugation of cell surface), as observed in the interphase amoebae after enucleation, and in the mitotic ones in which the nuclear material is dispersed. If so, the regulatory role that the nucleus plays in normal locomotion of interphase cells, might be interpreted as that of waste depot liberating the cytoplasm from the excess of substance which is needed for locomotion in an appropriate concentration, but if not removed after accomplishing its task, either as such either in a decomposed form, it would indefinitely accumulate, inhibit motion, and induce "bubbling".

If the author interprets correctly the postulates of the "tail organizers" theory, it would offer an explanation of some differences of behaviour between the anterior and posterior anucleate fragments, as described in this study. The anterior fragments would cease movement because of exhausting the reserve of tail organizer substance present in the cytoplasm, and the posterior ones, on the contrary, because of accumulating it in the excessive amount and "bubbling". This difference might account for the smooth appearance of the anterior fragments and the corrugation of the posterior ones, after they cease movement.

However, the acceptance of the "tail organizers" theory presents much more inconvenience, for the following main reasons:

(1) It offers no unequivocal explanation of difference in the motility times of the anterior and posterior anucleate fragments.

(2) It relates the origin of the regulatory substance to the morphological tail (the uroid) which is directly contradicted by the present demonstration that the motile capacities of fragments are reversed by the reversals of the cytoplasmic stream, earlier than any changes in external morphology take place.

(3) It postulates the regulation of motility based on a gradient of ATP production and it includes the presumption that the contraction

takes place when the critical concentration of ATP is attained. Such a concept of triggering the contraction has been abandoned long time ago by muscle biochemists, and there are no conclusive data available on its existence in other motile cells. [2]

(4) It would require a strict stability of the tail which is contradicted by the possibility of changing experimentally the main axis of motory polarity in amoeba (Grębecka 1977).

(5) It would predict a cessation of movement in amoeba after cutting out the posterior portion of its body: cells operated in that manner should behave as premitotic specimens in which the stock of the hypothetical substance in the tail, has been exhausted. This is disproved by author's experiments (unpublished before) consisting in multiple cutting out the successive tail segments of moving polypodial or monopodial amoebae. Such operations never had any significant influence upon the motility of dissected specimens. Even in monopodial forms of *A. proteus*, in which the resection of successively restored tails was repeated seven times in each individual, the only result obtained was a modification of proportions of their cylindrical body shape, and declining dimensions of the successive uroids, both effects being related to the gradual reducing of the cell membrane stock by cutting out the posterior extremely folded regions.

All these considerations convince the author to base rather on the results of Goldstein and Jelinek (1966) and on the views developed further by Goldstein (1973), and to part from the principle that the crucial point in the control of motory activity in interphase cells, should not be any deposition of remnants of the regulatory substance in the nucleus, but on the contrary, the emanation of the regulatory factor in its active form from the nucleus. This concept explains not only the necessity of nucleus for normal locomotion, but also the motility of fragments cut from dividing amoebae in which the nuclear material is dispersed.

The hypothesis of Goldstein and Jelinek (1966) has to be supplemented by stating that the mechanism of distribution of the regulatory substance over the cell interior, after its excretion from the nucleus, consists in spreading it out by the cytoplasmic stream which, during normal locomotion, is flowing around the nucleus on its way from the rear body regions toward the front. This should mean

[2] Even Allen and Taylor (1975) who suspect the possibility of an ATP gradient along the body axis of amoeba, refer to higher ATP concentration needed for relaxation and not to that which is critical for contraction, and they stress that the real existence of such gradient in intact amoeba has not yet been demonstrated.

that a regulatory substance, if it is (1) emanating from the nucleus, (2) is transported by the cytoplasmic stream, and (3) is used up during locomotion, will manifest the following pattern of distribution: an important amount present between the nucleus and the advancing front, and an insignificant concentration or absence between the rear body edge and nucleus (Fig. 12).

If so, the difference in the times of motility between the anterior and posterior anucleate fragments finds a good explanation. Motility of such fragments, which are deprived of any fresh supply of the regulatory substance needed for locomotion, may depend only on its concentration present at the moment of dissection. The direction of the cytoplasmic stream carrying the substance is responsible for its deficiency in the posterior fragments, and for its presence in the anterior ones in a quantity depending on the rate of former nuclear excretion. A difference between the motility times of both kinds of anucleate fragments, should be therefore expected, at least during the first period after operation.

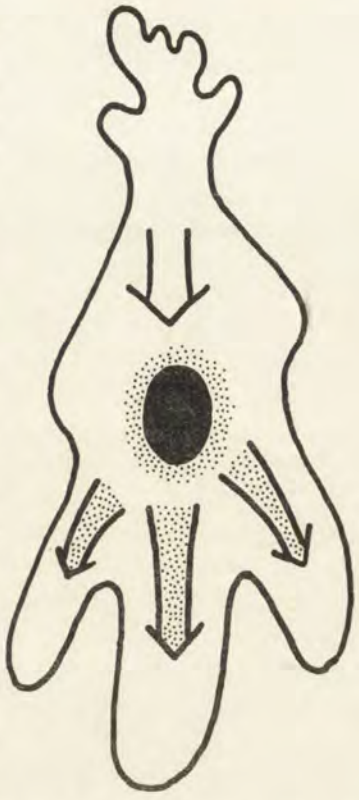

Fig. 12. Schematic presentation of the turnover of the hypothetical relaxatory agent emanating from the nucleus (dots), carried forwards by the cytoplasmic stream (dotted arrows), and directed back to the nuclear region from behind in an inactivated form (empty arrow)

The results obtained in this study confirm in full extent the concept proposed above. The difference in the motility times is not very great, it is to be measured in minutes, however, it is unequivocal in this respect that the anucleate fragments, if they contain the areas which were situated between the nucleus and the front of amoeba, will always preserve longer their capacity to move. Further arguments in favor of the hypothesis of nuclear origin of the substance regulating movement, and of the cytoplasmic stream playing role of its carier, are brought by bisection of amoebae with spontaneous reversal of streaming toward the uroid and of the V-shaped specimens manifesting before the operation a period of oscillatory reversions of streaming. Each fragment containing the regions into which the reversed streaming has been directed from the perinuclear zone, behaves merely like an anterior fragment cut from

a normally moving individual. This effect may be obtained equally well by means of artificial reversion of streaming induced by injection of one or two droplets of paraffin oil. In all cases the change of streaming direction leads to the corresponding change of motory capacities of resected anucleate fragments, which proves that in fact the cytoplasmic stream is responsible for the intracellular distribution of the regulatory factor which controls the motory activity.

As far as the question is concerned of the mode of the control effected, some suggestions are offerred by the net difference of shape and of surface conformation between the anterior and posterior anucleate fragments. Preparations poor in the nuclear regulatory factor, such as enucleated amoebae investigated by precedent authors and the posterior anucleate fragments described in this study, assume quickly the form of corrugated spheres [3] with the peripherial zone strongly folded, which suggests an uninhibited contraction. The anterior anucleate fragments and other fragments enriched in the regulatory substance brought from the nucleus by the cytoplasmic stream, exhibit on the contrary, a smooth surface during quite significant period after the cessation of movement, which seems to indicate that the generalized contraction is for a certain time inhibited.

This interpretation applies also for the intact amoebae, in which a posterio-anterior gradient of membrane folding has been found (Czarska and Grębecki 1966 and Stockem et al. 1969) as well as the parallel gradient of contraction-extension (Grębecka and Grębecki 1975). This means that during normal locomotion the extension of anterior body regions and unfolding of their peripherial layer are probably also related to their downstream position in respect to the nucleus.

It seems therefore possible to suppose that, in general, the substance emanating from the nucleus is in some way responsible for the relaxation in amoeba cell. [4]

As far as the chemical identity of this hypothetical nuclear relaxator is concerned, one can only refer to Goldstein (1973) who reports that some not yet definet unstable proteins are migrating in amoeba from the nucleus to the cytoplasm and backwards. Their unstability might explain the relatively quick cessation of movement in the absence

[3] The fact that some anucleate fragments of amoeba may assume the corrugated appearance has already been observed by Hofer 1890 (quoted after Lorch 1973).

[4] It would seem interesting to relate the postulated relaxatory role of nucleus to the lack of adhesion in anucleate fragments, in particular in the light of ideas developed by B. M. Jones (1966) and P. C. T. Jones (1966) about the importance of relaxation for cell adhesion, and of localization of the attachment zone in intact *A. proteus* by Grębecki (1976). This suggestion remains open to experimental testing.

of nucleus, and in the intact amoebae it could account for the lack of active relaxator behind the nucleus (upstream), as postulated in this study. Their unstability may also simply signify that the relaxing factor has to undergo some change to induce contraction. After accomplishing this task it should be removed from the contraction site and recover its former state elsewhere, which perhaps corresponds to the stage of backward migration to the nucleus effected by the proteins discovered by Goldstein.

RÉSUMÉ

Les cellules de l'*Amoeba proteus*, formes polypodiales et monopodiales, étaient sectionnées juste avant ou juste derrière le noyau, pour obtenir respectivement des fragments sans noyaux antérieurs et postérieurs. Les fragments antérieurs préservent leur mobilité pendant une période sensiblement plus longue que les postérieurs. En plus, les fragments sans noyau antérieurs présentent une surface lisse, tandis que chez les postérieurs elle dévient immédiatement plissée. Les inversions spontanées ou artificielles du courant cytoplasmique résultent en ce que les fragments postérieurs se comportent comme les antérieurs. Les capacités motrices et la conformation de la surface d'un fragment ne dépendent pas de sa position par rapport à la morphologie extérieure de l'amibe, mais de la position qu'il occupait avant l'opération par rapport au noyau: en aval ou en amont du courant cytoplasmique. Cette régularité s'accorde mal avec la théorie des „tail organizers" de Goldacre et Lorch, mais par contre elle permet de suggérer que ce sont les protéines labiles, dont les migrations dans les deux senses entre le noyau et le cytoplasme ont été découvertes par Goldstein, qui jouent le rôle du facteur régulateur de l'activité motrice en agissant comme agents rélaxateurs, et que ce facteur est distribué dans l'intérieur de la céllule par le courant cytoplasmique.

REFERENCES

Allen R. D. and Taylor D. L. 1975: The molecular basis of ameboid movement. In: Molecules and Cell Movement. Raven Press, 239–258.

Becker E. R. 1926: The role of nucleus in the cell functions of amoebae. Biol. Bull. 50, 382–392.

Brachet J. 1955: Recherches sur les intéractions biochimiques entre le noyau et le cytoplasme chez les organismes unicellulaires. Biochim. Biophys. Acta, 18, 247–268.

Clark A. M. 1942: Some effects of removing the nucleus from amoebae. Austral. J. Exp. Biol. Med. Sci., 20, 240–247.

Clark A. M. 1943: Some physiological functions of the nucleus in amoeba, investigated by micrurgical methods. Austral. J. Exp. Biol. Med. Sci., 21, 215–220.

Clark A. M. 1944: The responses of enucleated amoeba to stimuli. Austral. J. Exp. Biol. Med. Sci., 22, 185–196.

Czarska L. and Grębecki A. 1966: Membrane folding and plasma-membrane ratio in the movement and shape transformation in *Amoeba proteus*. Acta Protozool., 4, 201–239.

Dawson J. A. and Belkin M. 1928: The digestion of oils by *Amoeba dubia*. Proc. Soc. Exp. Biol. Med., 25, 790–793.
Dawson J. A. and Belkin M. 1929: The digestion of oils by *Amoeba proteus*. Biol. Bull., 56, 80–86.
Goldacre R. J. 1961: The role of the cell membrane in the locomotion of amoebae, and the source of the motive force and its control by feedback. Expl. Cell Res. (Suppl.), 8, 1–16.
Goldacre R. J. and Lorch I. J. 1950: Folding and unfolding of protein molecules in relation to cytoplasmic streaming, amoeboid movement and osmotic work. Nature, 166, 487–499.
Goldstein L. 1973: Nucleocytoplasmic interactions in amoebae. In: The Biology of *Amoeba*, Academic Press, 479–504.
Goldstein L. and Jelinek W. 1966: A comparison of the movement of enucleated fragments from interphase and mitotic amoebae. Expl. Cell Res., 43, 51–55.
Grębecka L. 1977: Changes of motory polarization in *Amoeba proteus* as induced by oil injections. Acta Protozool., 16, 107–120.
Grębecka L. and Grębecki A. 1975: Morphometric study of moving *Amoeba proteus*. Acta Protozool., 14, 337–361.
Grębecki A. 1976: Co-axial motion of the semi-rigid cell frame in *Amoeba proteus*. Acta Protozool., 15, 221–248.
Jeon K. W. 1968: Nuclear control of cell movement in amoebae. Nuclear transplantation study. Expl. Cell Res. 50, 467–471.
Jones B. M. 1966: A unifying hypothesis of cell adhesion. Nature, 212, 362–365.
Jones P. C. T. 1966: A contractile protein model for cell adhesion. Nature, 212, 365–369.
Kalisz B. and Korohoda W. 1976: Experimental study on locomotion of *Amoeba proteus*. I. Movements in the nucleated and anucleated fragments of the amoebae after removal of the part of their cytoplasm. Acta Protozool. 15, 345–361.
Lorch I. J. 1969a: The rate of attachment to the substratum: A study of nuclear-cytoplasmic relationship. J. Cell Physiol., 73, 171–177.
Lorch I. J. 1969b: A comparison of the movement of amoebae enucleated at different stages of their life cycle. J. Cell Biol., 43, 82 a.
Lorch I. J. 1973: Some historical aspects of amoeba studies. In: The Biology of *Amoeba*, Academic Press, 1–36.
Lorch I. J. and Danielli J. F. 1950: Transplantation of nuclei from cell to cell. Nature, 166, 329–330.
Lorch I. J. and Danielli J. F. 1953: Nuclear transplantation in amoebae. II. The immediate results of transfer of nuclei between *Amoeba proteus* and *Amoeba discoides*. Quart. J. Microcs. Sci. 94, 461–480.
Marsland D. 1933: The site of narcosis in a cell; the action of a series of paraffin oils on *Amoeba dubia*. J. Cell. Comp. Physiol. 4, 9–33.
Ord M. J. 1968: The viability of the anucleate cytoplasm of *A. proteus*. J. Cell Sci. 3, 81–88.
Stockem W., Haberey M. and Wohlfarth-Bottermann K. E. 1969: Pinocytose und Bewegung von Amöben. V. Mitteilung. Konturveränderungen und Faltungsgrad der Zelloberfläche von *Amoeba proteus*. Cytobiologie, 1, 37–57.

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EXPLANATION OF PLATES I–VI

1–6: Example of dissection of a polypodial amoeba in the posterior nucleate fragment (up on the picture) and the anterior anucleate one (lower). The anterior part without nucleus changes actively its position from the 1st up to the 3rd min after the operation, then at the interval between the 3rd and the 8th min it still manifests the coordinated intracellular streaming and pseudopodial activity, and at the 10 min stage it forms a clod with smooth surface. Its final rounding up is not shown

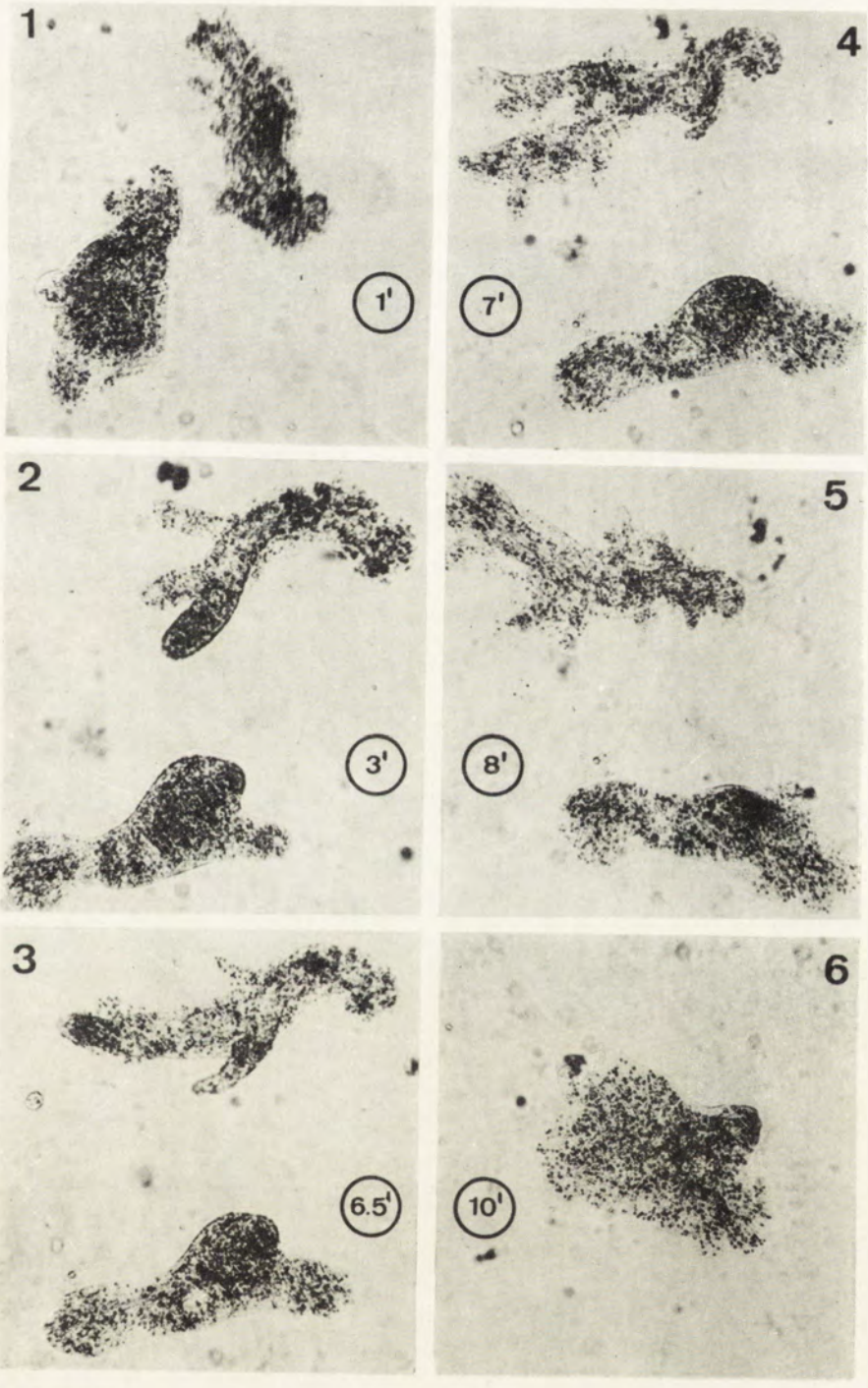
7–12: Example of dissection of a polypodial amoeba in the anterior nucleate (upper right) and the posterior anucleate fragment (lower left). The posterior part without nucleus does not locomote, manifests neither coordinated intracellular streaming neither polarized pseudopodial activity. The only process seen over the whole sequence, lasting for about 2 min, is its gradual rounding up and strong corrugation of its surface

13–18: Example of two dissections, effected at the interval of 1 min, separating from polypodial amoeba two successive posterior anucleate fragments. The distal fragment shows no activity but immediate rouding up and corrugation, whereas the second, more proximal one, manifests regular pseudopodial activity and smooth surface from the moment of cutting it off up to the end of the sequence, i.e., for 3 min

19–21: Cutting out the posterior anucleate fragment (left) from polypodial amoeba in which, just before the operation, the inverse cytoplasm streaming started to invade the uroid. During 8 min after the operation, the posterior anucleate fragment and the anterior nucleate one, do not show any significant difference in their motory behaviour

22–27: Example of dissection of an artificially monopodial amoeba obtained by injection of a paraffin oil droplet. The anterior anucleate fragment with the oil cap preserves the motory capacity during first 5 min, and at the 7 min stage when it already rounded up, it still keeps its surface smooth

28–33: Example of bisection of polypodial amoeba in which the cytoplasmic stream has been separated into two branches by injection of two oil droplets (stage 0'). The anucleate part (right on the picture) manifests regular motory activity at the stages 1' and 2.5', it slows down and becomes a clod with the surface still smooth at the stage 6', and only 11 min after the operation it begins to corrugate (at the same stage the nucleate part lost its oil cap and recovered the regular shape).



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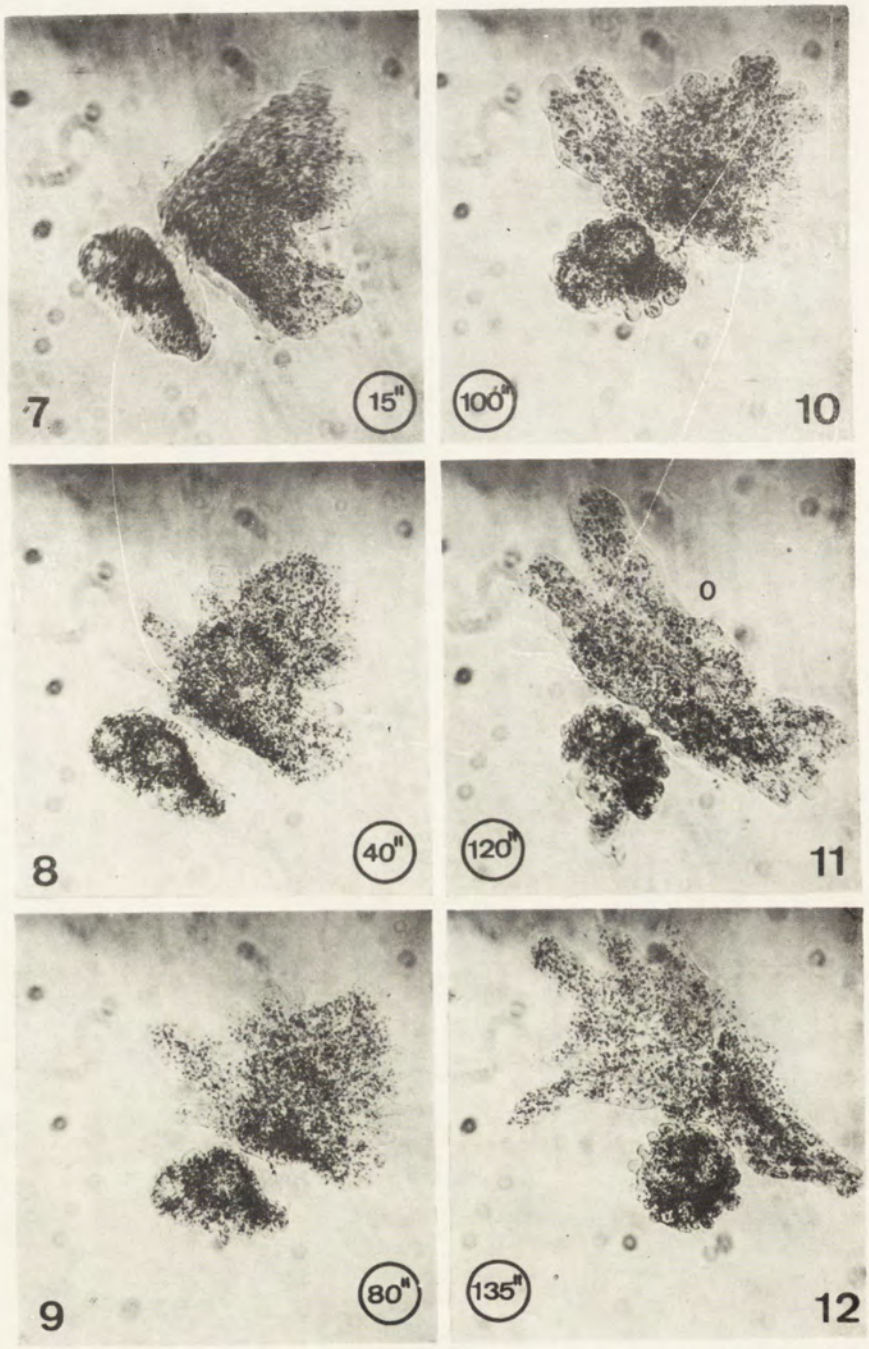

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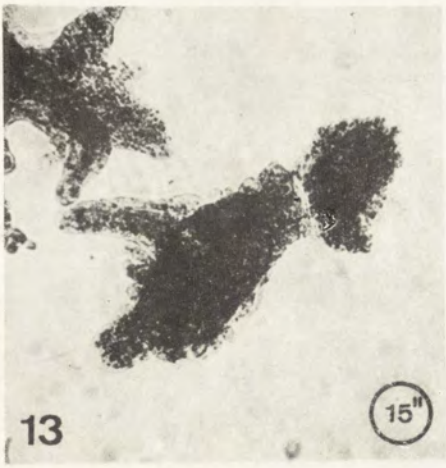

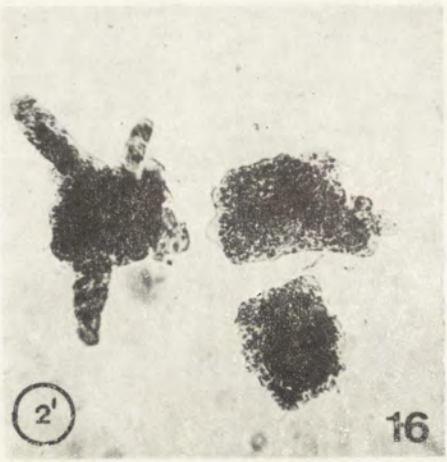

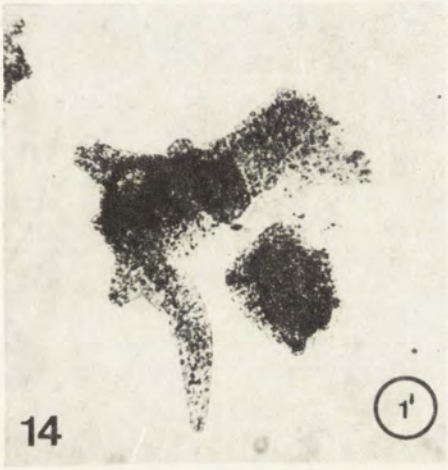

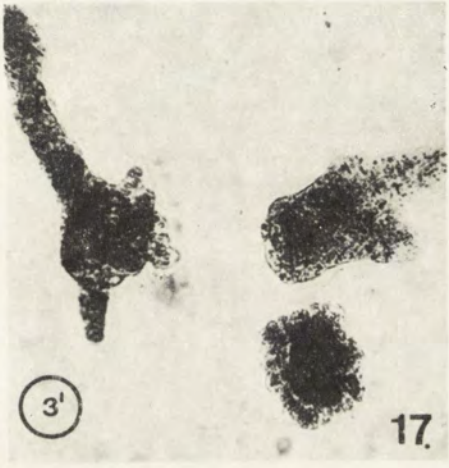

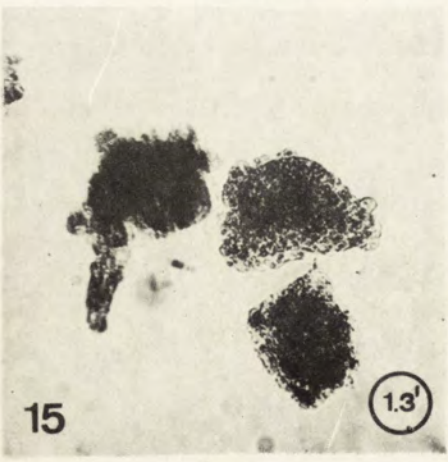

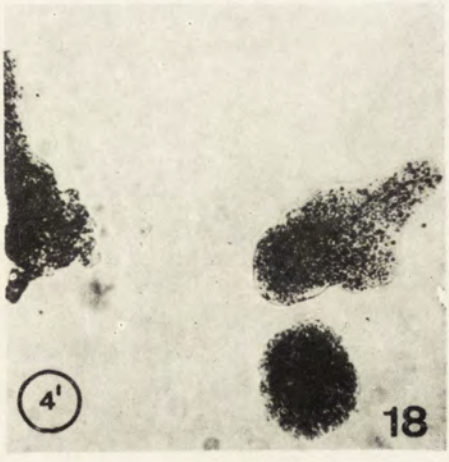

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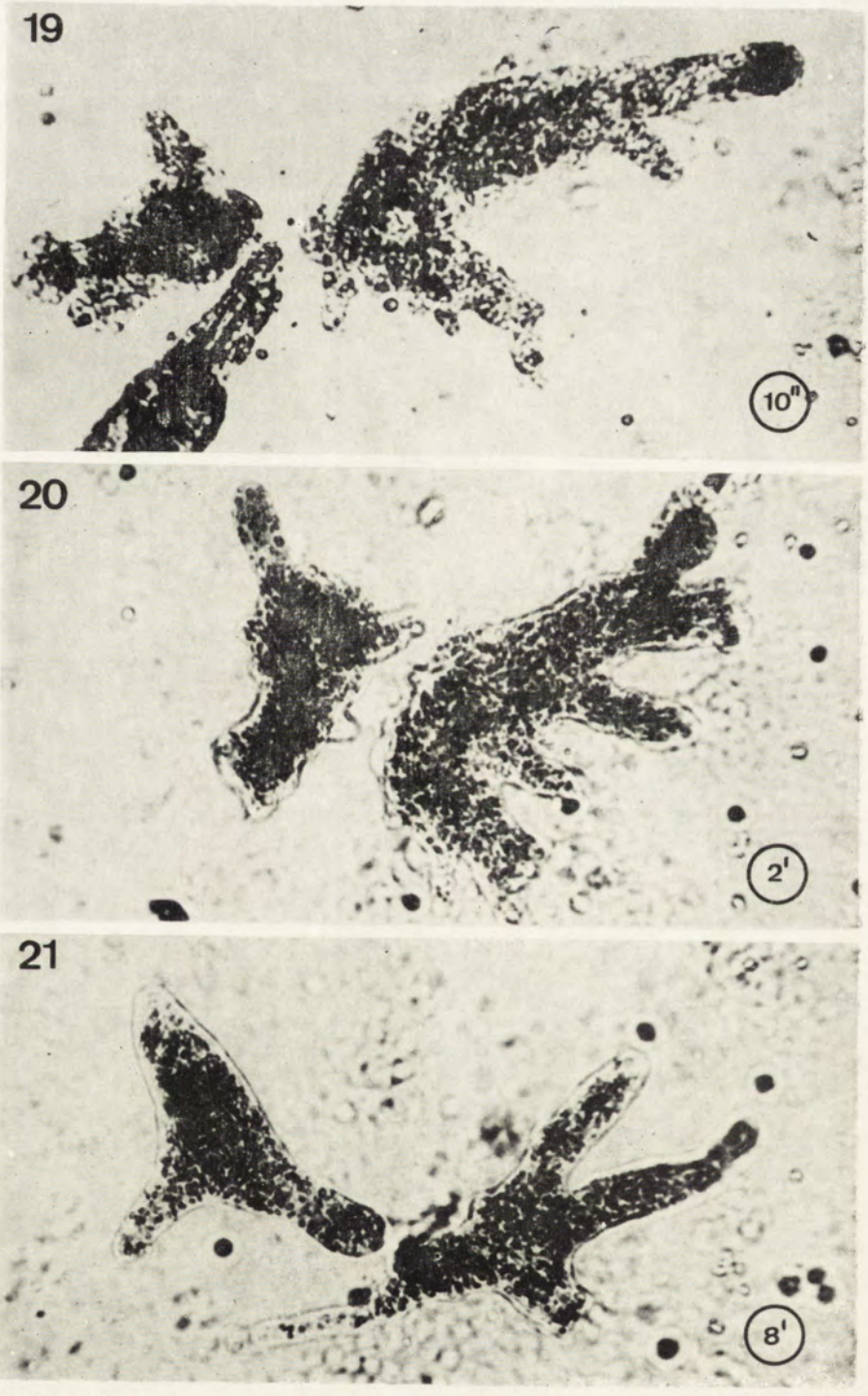

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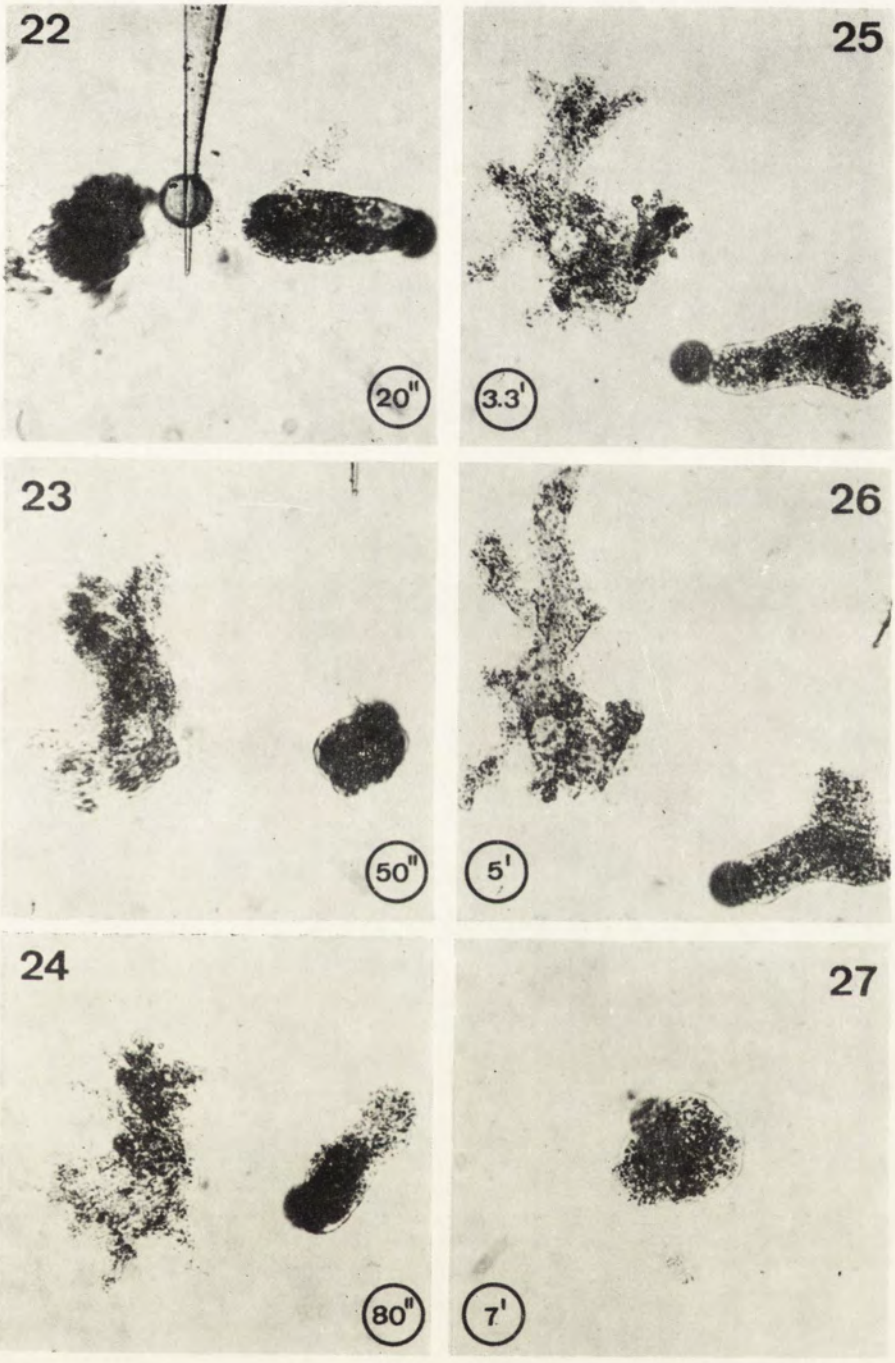

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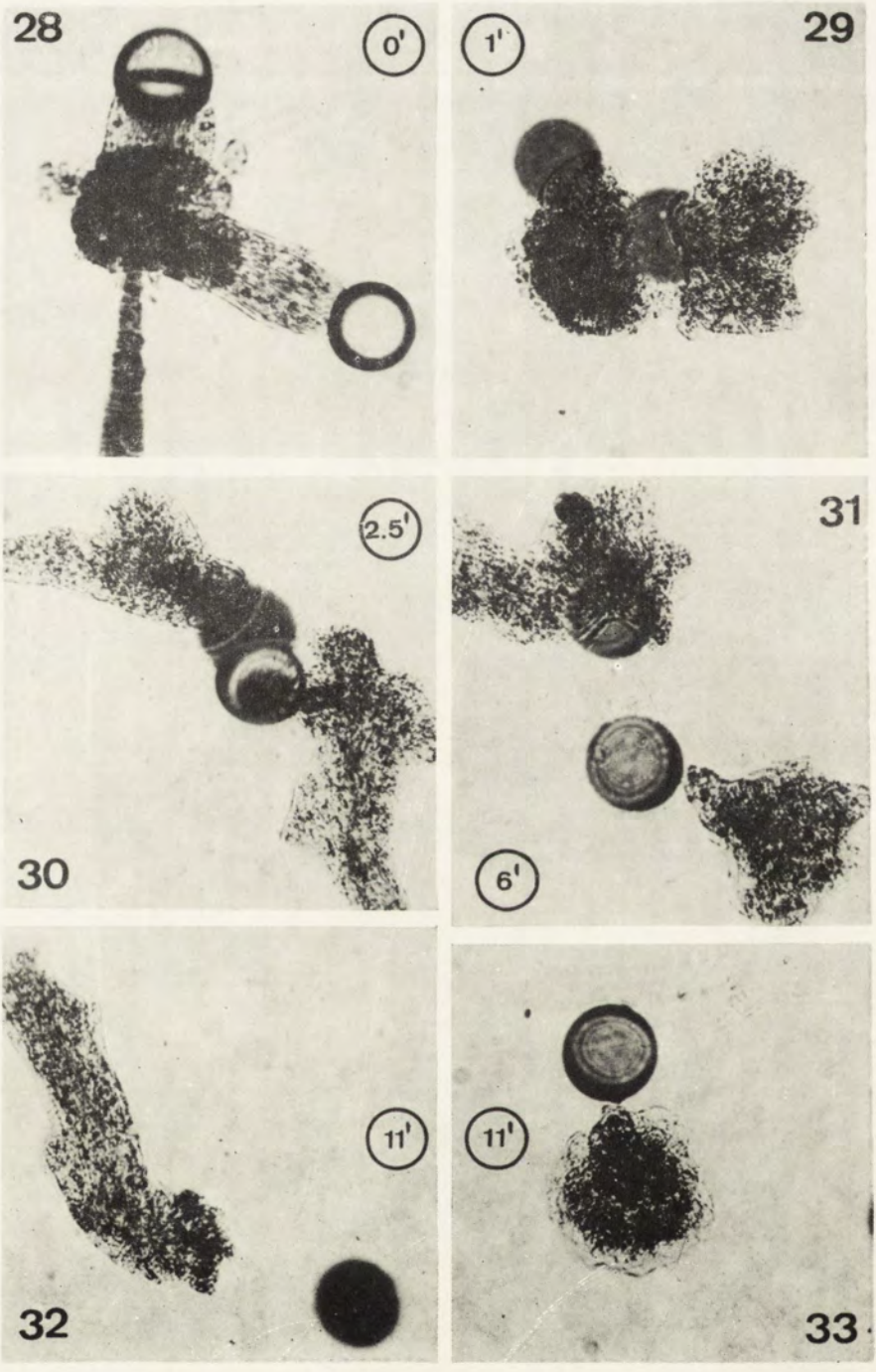

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Changes of Motory Polarization in *Amoeba proteus* as Induced by Oil Injections

Synopsis. Droplet of paraffin oil injected into polypodial amoeba against the inner face of its membrane initiates extension of a huge leading pseudopodium with oil cap, and reorganizes the former cell polarity. Injections into the uroid inverse completely the motory axis. In monopodial specimens different cases of competition between the oil cap and the "hyaline" cap are obtained, but never a complete and definite inversion of polarity. Two successive equilibrated oil injections into polypodial specimens prove that the cap formed earlier is dominating. Equilibrated double-cap amoebae may recover by extending lateral pseudopodium in the middle of their elongated body. It is suggested that in the first phase the oil injection distends the peripherial cell layers and directs the cytoplasm streaming into the breach. Probably, the streaming carries a regulatory substance emanating from the nucleus, which relaxes the cell cortex, reinforces and stabilizes the new direction of endoplasmic flow, and eventually establishes a new contraction–relaxation gradient and a new motory axis.

In normally moving polypodial amoebae the cytoplasm sometimes manifests spontaneous reversals of its streaming direction. This may finally lead to a corresponding change of the general direction of cell locomotion. The inversion of cytoplasm flow is also followed by change in the dynamic morphology of amoeba: the former tail transforms in the new front with prominent advancing pseudopodia covered with smooth membrane, and the former front reduces its size, shrinks and collapses, assuming the shape typical of a tail. Careful observation leads to the conclusion that the cytoplasm streaming reversal is the primary event initiating the whole sequence. [1]

[1] The statements above apply to the phenomenon of complete reversal of cell polarity, and not to that of a simple turn modifying the direction of locomotion. In the first case the reversed flow starts at the former tail and the pattern of two divergent streamings is temporarily manifested, whereas in the second case the former front may react first and two opposite streamings converge. The difference between the divergent and convergent streaming effects has already been pointed by Mast (1926). The divergent streamings were also described during oscillatory changes of polarity in amoeba (Goldacre 1956) and at the initial stage of reversing the flow direction in pseudopodia (Allen 1961, 1973).

These common observations confirm, on the one side, the dynamic character of amoeba macromorphology, and on the other, they put in question the possibility to locate the contractile activity of amoeba in any fix cell region, definite and stable in time. This last statement needs some additional comments. It is not directed neither against localizing the contractile capacity in one of the micromorphological body layers, neither against the regular distribution of the contraction–extension gradient along the temporary axis of locomotion. It means only that all the body parts, as well the uroid as the advancing or withdrawing pseudopodia, are omnipotent and may be in the appropriate conditions induced to form either a new contracting tail, either a new extending front of moving amoeba. One could imagin an amoeba, freely moving in an absolutely uniform environment, to be experimentally modeled by application of controlled local stimuli, and finally reshaped in the way completely independent from the initial pattern.

Such ideal experimental conditions remain a pure abstraction, and we actually work on amoebae which move in their normal medium, being exposed to certain number of uncontrolled stimuli which permanently shape and reshape their dynamic morphology. Under such conditions, the plasticity of amoeba macromorphology and motory polarization may be tested only by means of applying stimuli which are relatively strong, strictly localized, and acting for a long time.

All these requirements are met by oil droplets of appropriate volume which are injected with glass micropipettes into the cell interior against the inner face of its wall. The paraffin oil is physiologically harmless, and the association of an oil droplet with amoeba cell is lasting enough long time to observe and to record photographically the exerted effects. The presence of an oil droplet under the cell membrane may be considered as a kind of positive stimulus, because the cytoplasmic stream is continuously oriented in its direction.

The motory effects of oil drops attached to the membrane of amoebae: formation of oil caps initiating huge pseudopodia and transforming the whole organism into an artificially monopodial form, were described first by Dawson and Belkin (1928) in *A. dubia*, and confirmed by Marsland (1933) in the same species. These authors applied the oil externally. Dawson and Belkin (1929) and Marsland (1933) were unable to obtain artificial monopodial specimens in *A. proteus*. The effective method of obtaining monopodial *A. proteus* with permanent oil cap has been elaborated only by Goldacre (1961), and it consisted in injecting the oil into the cell interior in such manner that the droplet becomes associated with the inner surface of the membrane. Goldacre (1961) reported briefly the immediate motory

effects of such injections, and the further behaviour of amoebae subjected to this operation was described by Czarska and Grębecki (1966).

Material and Methods

Polypodial and monopodial forms of *Amoeba proteus* were used for experiments. The cultures were maintained in Pringsheim medium. Colpidia grown in yolk cultures were added as food.

Intracellular injections of the paraffin oil were done with micropipettes drawn in De Fonbrune microforge, and operated by means of De Fonbrune micromanipulator. All the operations, and further observation of amoebae, were done on specimens kept in drops of Pringsheim medium and closed in De Fonbrune oil chambers. Pictures were taken either in the bright field either in the dark field obtained with the PZO polarization-interference microscope.

Results

Single Injections into Polypodial Amoebae

The Pl. I shows five successive stages of behaviour of a polypodial specimen which received injection of oil droplet into its uroid. The pictures were taken at intervals of 2–3 s, just necessary to rewind the camera shutter. The sequence shown in the Pl. I 2, 3 and 4 proves that the reversal of the cytoplasmic stream is a very rapid, if not instantaneous, phenomenon which is the more so striking that the reorientation of the streaming towards the oil drop injected into the uroid, necessitates the complete inversion of its direction.

This result is inconsistent with the theory of the semi-permanent tail being the only site of the contraction force driving the amoeba, as postulated by Mast (1926), Goldacre and Lorch (1950) and Goldacre (1956). According to this view one should expect either the cessation of streaming after the blockade of tail by the oil either its acceleration due to the mechanical stimulation, but never its reorientation in the opposite direction.

The change of the cytoplasm streaming direction is followed by corresponding changes in the shape of amoeba. The former tail assumes the form of a smooth pseudopodium headed by the oil cap (similar to the front of a natural monopodial amoeba bearing its "hyaline" cap). The former frontal regions shrink, fold, and their edge becomes corrugated as in the typical tail of any other moving specimen. Comparison of pictures presented in the Pl. I 1 and 5 shows the extent of these changes only at their initial stage. One can conclude that the ap-

propriate stimulation by oil injection results first in the inversion of endoplasm flowing direction, and thereafter in the inversion of other aspects of cell polarity: of its general contraction–extension gradient (Grębecka and Grębecki 1975), of the surface folding gradient (Czarska and Grębecki 1966, Stockem et al. 1969), and finally of the orientation of the main axis of locomotion.

The Pl. II 6 shows another polypodial amoeba with the oil droplet injected into the uroid, at the stage comparable to that of the Pl. I 5, but recorded by time-exposure technique in the dark field. The micrograph proves that amoeba was still keeping its initial general shape, with the former advancing pseudopodia seen in the upper part of the picture, and the former tail down. The anterior body part maintains its usual form and the pseudopodia exhibit the surface smooth, but they do not move forwards any more: all the cytoplasmic granules inside them were recorded as bright spots what means that the former front became stationary, at the moment of taking this picture. On the contrary, all the cytoplasmic granules in the former tail produced a dense pattern of parallel streaks directed backwards, i.e., toward the oil drop. The picture presents the directly recorded evidence that the inversion of the cytoplasmic stream, provoked by the oil injection, is the primary event which precedes the subsequent macromorphological reshaping of amoeba and the resumption of locomotion in new direction.

Oil droplets injected into other parts of the cell exert similar effects (Pl. III 7). Depending on the site of injection, the former tail either preserves its role either it becomes distended and substituted in its functions by another body region.

The further fate of such amoebae depends on the stability of their association with the oil droplets. As long as this association is maintained, amoeba moves forwards exactly in the same manner as a normal monopodial specimen. Even the fountain streaming is clearly seen in its frontal part behind the leading cap. When the oil cap is lost, the individual keeps still the same direction of motory polarization which has ben imposed earlier by the oil injection.

This phenomenon may be seen in the Pl. III 7–8 in which the first picture shows the formation of artificially monopodial form from a polypodial specimen, immediately after oil injection, and the next picture presents the same individual nearly 10 min later, when it was loosing the oil drop but still preserved the same direction of motory axis as it was induced by the former oil cap.

Presence of the cell nucleus is necessary to stabilize the first effect exerted on the cell polarity by oil injection. The oil drop injected into an anucleate anterior fragment of polypodial amoeba is capable to

polarize it at the initial stage (Pl. III 9, picture taken during the 2nd min after bisection and injection), but without the nucleus the polarity cannot be indefinitely sustained, and the fragment transforms into a smooth sphere, even before loosing the oil droplet (Pl. III 10, nearly 10 min later).

Single Injections into Monopodial Amoebae

As it is generally known, in *A. proteus* permanently monopodial specimens represent a peculiar case of extremely, or perhaps "excessively", developed motory polarization. The surface conformation gradient is also very well expressed in them: the cell membrane folding is strongly pronounced but strictly limited to the uroid and the closely adjacent posterior portion of trunk, whereas the main part of the body constitutes just one permanent pseudopodium with smooth surface, leaded by a structure called earlier the hyaline cap but thought to be a vacuole by Korohoda and Stockem (1975). The question of natural or pathological origin of such forms is beyond the scope of this study and will be later approached by the author elsewhere. In the present work the monopodial amoebae were simply selected from the cultures, when spontaneously appearing in them, and used for experiments as subjects particularly interesting because of their strong motory and morphological polarization.

When testing the behaviour of polypodial amoebae it is relatively easy to estimate the volume of the oil to be injected for producing the effect desired. This is much more difficult in monopodial specimens, because their polarity depends on the "hyaline" cap which is a factor strictly localized in the cell but hardly estimable as to the nature and the intensity of its influence. Without any notion of the character and value of intracellular force maintaining the original polarity of a monopodial amoeba, one cannot predict what volume of oil injected would be needed to counteract it and to inverse the direction of cytoplasmic stream. Plates IV, V and VI demonstrate that the results of injections may vary, depending on the relative size of oil droplets introduced.

Plate IV presents the results of injecting the paraffin oil in the amount which would be sufficient to re-polarize definitely a polypodial specimen of similar size. In the monopodial amoebae however, such injection produces only a temporary dominance of streaming directed to the oil droplet (Pl. IV 8–9). Nearly 20 s later (Pl. IV 10) an unstable equilibrium is established between two branches of intracellular streaming: that directed toward the "hyaline" cap and that directed to the oil cap. In the next phase, the branch of stream flowing toward the original

frontal cap restores its dominance (Pl. IV 11) which results in repulsing the oil drop into the tail region (Pl. IV 12–13). Such oil droplet attached to the uroid is eventually lost after a certain period (not recorded on pictures). It may be concluded that the size of oil droplet injected in this experiment was sufficient to induce a temporary local inversion of the cytoplasm streaming, but the influence of the native frontal cap was so strong that the cell, after brief perturbations, re-established its original polarization.

More important injections of the paraffin oil into the monopodial specimens, as shown on the Pl. V 14–24, result in the dominance of the huge oil cap which pulls the whole cell body, including its original front with the "hyaline" cap, and finally forces the amoeba to round up. The spherical form which is obtained, obviously cannot locomote, but the intracellular streaming persists inside it for long time and is directed toward the oil drop. Conclusion may be drawn that the inversed polarization is attained as far as the intracellular streaming is concerned, but beside this, the presence of original "hyaline" cap at the opposite pole prevents the formation of a new tail in its place. Therefore, the regular gradients of contraction-extension and folding-unfolding in the peripherial cell layers cannot be fully established in the new direction. As result, the inversion of the intracellular streaming polarity is not followed by the corresponding reorientation of the locomotory axis, but rather by its effacement.

Intermediate effects were brought by moderate oil injections, when they happened to be equilibrated enough, just to compensate the influence of original frontal cap. Such injections produce amoebae with clearly pronounced but defective and periodically changing direction of polarization. One pole of the elongated and smooth cell body is formed by the "hyaline" cap and another by the oil cap. The cytoplasm streaming alternately predominates either in one either in the second direction, and produces frontal fountains either at one either at another pole. Such unstable equilibrium state may continue even for several hours, and then some individuals finally round up. In many other cases, amoebae form a lateral pseudopodium, mostly halfway between the "hyaline" cap and the oil cap (Pl. VI 29–30), which becomes the new advancing front. As result, as well the oil drop as the frontal vacuole are shifted into the new posterior region. Then usually the oil droplet is detached, but the native vacuole reverses the cell polarity once again and resumes its former functions of the leading frontal cap. Recovery of the polypodial form of amoeba by loosing both the oil and the "hyaline" caps cannot be *a priori* excluded as a possibility, but it has never been observed by the author.

It should be stressed in conclusion that in natural monopodial amoebae the so called "hyaline" cap plays a role of very efficient positive stimulus, of the attractant permanently present inside the cell, automatically deciding about the position of the front and, consequently, about the direction of locomotory axis. Nevertheless, even in a so strongly polarized form of amoeba, the polarity may be at least partially or temporarily disturbed, by competition of injected oil drops.

Double Injections into Polypodial Amoebae

Third group of experiments concerned polypodial forms of *A. proteus* in which the polarity changes were induced by injection of two separate oil drops. Results of such experiments depend as well on the size relationship between both oil caps obtained, as on the time interval between two successive injections.

When the volumes of both injected droplets are different, the cytoplasmic stream flows, as a rule, towards the larger cap, and the smaller one is, sooner or later depending on the size difference, repulsed to the posterior body end. The size effect appears in the most clear form when both injections succeed one another at the shortest possible interval. Of course, at the first moment after each injection the cytoplasm starts flowing toward the injected drop, independently from its size, but very soon this autonomy of oil caps disappears. Amoeba elongates and the cytoplasmic stream flows toward the bigger drop, and the smaller one remains just weakly attached to the posterior body region and finally eliminated. The initial autonomy of both caps is maintained for a few seconds only, if the size of oil drops is very different, but it lasts much longer if both oil injections were more similar in volume.

Equally important is the anteriority factor. The initial autonomy phase is extremely brief in the smaller oil cap which has ben produced by the second injection, but it becomes several times longer if the smaller drop was the first to be injected. Two oil droplets slightly different in size may behave as perfectly equivalent if there was a slight difference in the time of their injection, in favour of the smaller one. On the contrary, two droplets of equal volume are not equilibrated if they were not injected immediately one after another: the second one is subordinated, repulsed into the tail, and finally eliminated. [2] This last

[2] This might explain why Goldacre (1961) reported that it is not possible to produce amoebae with two active oil caps, and that the second injected drop fails to induce any motory effects and is finally detached from the tail region. In the present experiments the author also failed to obtain amoebae with two independent oil caps, but only in such cases when the second drop was too small or when it was injected too late.

situation is in large extent similar to the case of monopodial amoebae in which the oil injections fail to reverse completely and definitely the former polarization controlled by the "hyaline" cap.

It should be concluded in general that, in order to obtain a balanced competition between two oil drops injected into polypodial amoeba, special care should be taken to inject two droplets equal in volume and to execute both injections at the shortest possible time interval.

The Pl. IX presents the results of injection of two relatively large droplets, similar in volume but not identical, immediately one after another. At the initial phase (Pl. IX 53–54) the cytoplasm streaming flows toward both oil caps in uniform manner. In the next period (Pl. IX 55–56), the slightly bigger cap situated on the left in respect to amoeba axis, becomes dominant, and the branch of streaming flowing in its direction acquires more importance. Finally, the subordinated oil drop does not attract any cytoplasm streaming any more (Pl. IX 57–58), and at the same time it is gradually shifted more and more toward the uroid (Pl. IX 56–59). However, the size difference between both oil drops was not enough significant to provoke the definite elimination of the smaller one. As result, both drops remain long time attached to the opposite poles of amoeba, and the sporadic reversals of the cytoplasmic stream are manifested (Pl. IX 60–61). The volume of both oil drops used in this experiment was relatively large in respect to the cell size, and in such a case amoeba finally rounds up (Pl. IX 62).

In the experiment shown in the Pl. VIII the oil droplets were relatively smaller, more precisely equilibrated in volume, but the drop seen in the lower part of the picture has been injected 10 s earlier than the upper one. For that last reason the oil caps initiated two branches of streaming, well separated from the very beginning, and gradually assuming opposite orientations. In consequence, the former uroid became the middle part of distended body. Lower branch, the earlier one, is dominating initially in volume (Pl. VIII 42–47) and thereafter in volume and length (Pl. VIII 48–52). In this experiment the size of oil droplets was insufficient to force the amoeba to round up, and it kept the elongated form for several hours. In many cases, at the middle body region of such elongated specimens, the cytoplasm streaming is absent and the surface layer is folded in the similar manner as in the uroid of normal amoebae (Pl. X 68).

There exists only one manner to recover the normal polarity by amoebae distended between two equilibrated oil droplets: to establish a new locomotory axis perpendicular to the line connecting both caps. An example of this common phenomenon is given in the Pl. VI and VII. The Pl. VI 32–34 shows the first unsuccessful tentative to extend

a lateral pseudopodium, in the region situated close to one of the oil drops. Another lateral pseudopodium is formed later at the body middle (Pl. VII 35–37). It eventually becomes the new advancing front of amoeba, whereas the opposite body wall contracts, pulls both oil drops together, and becomes the new tail (Pl. VII 38–41). In this way the recovery has been accomplished, not by dominance of one of two opposite and competing orientations, but by escaping in a completely different and new direction.

Another example of initial stage of this phenomenon is given in the Pl. VI 31. The amoeba shown in the Pl. X 67 completely recovered its usual polypodial form and pattern of locomotion, but it still drags behind its uroid two former oil caps.

The same manner of restituting the normal shape and locomotion is observed in amoebae with three attached oil droplets (Pl. X 63–66). Two of them, equal in size and injected immediately one after another, established two competing poles, and their equilibrium could not be disturbed by the third oil drop injected later, in spite of its more important volume. Also this equilibrium has been broken by formation of new lateral pseudopodium.

Discussion

The present experiments cannot explain how the oil cap induces its motory effects, and the main purpose of this study was different: to examin the competition between two oil caps or between the oil cap and the native "hyaline" cap. That means that the oil caps were used only as appropriate stimuli, enough strong to change the original natural cell polarity or the polarity established before by an earlier injection. Therefore, the question of the mechanism of oil action will be left out of consideration, but the possible mechanism of maintaining the cell motory polarity, as suggested by the present author elsewhere (Grębecka 1977), will be confronted with the phenomena of caps competition.

In normally moving polypodial amoebae the main axis of locomotion follows the direction of the contraction-extension gradient, and therefore one can define its both extreme ends as the contracting pole and the extending pole (or more exactly, one can speak about multiple contracting and multiple extending poles, with temporary domination of one pole of each kind). However, each body region is capable to contract and to expand in response to external stimulation and/or to intracellular regulation of motory activity, what proves that both poles are facultative.

For that reason, the injection of oil droplet may easily and definitely change or even reverse the polarity of polypodial amoeba: the oil cap forms the new extending pole and the opposite body region is always apt to form the contracting pole. So, the new motory axis may be completely established.

The natural monopodial amoebae spontaneously arising in cultures have permanent and unique (not facultative) extending poles. The injection of oil droplet into such specimen forms in it a second extending pole, which may compete with the first natural one. However, it cannot reverse completely the motory axis, because there is no place where to form the contracting pole. Its prospective place, opposite to the oil cap, is occupied by the "hyaline" cap. Therefore, the absence of definite and full reversal of polarity by injections of oil into monopodial amoebae, may be explained by the impossibility of establishing a complete new motory axis.

The same explanation applies equally well to the fact that the second oil drop injected into polypodial amoeba usually fails to inverse completely the effects of the first drop injected earlier.

Under such conditions three eventualities are possible, and all the three were observed in the reality: (1) the oil droplet in monopodial amoeba, or the second droplet in the double injection experiment, is less efective and is eliminated, what leads to the recovery of the first polarity direction, (2) the oil cap and the "hyaline" cap, or two oil caps, are equilibrated and the cell is distended with alternating streaming, or it rounds up, what means that the polarity either remains abnormal either is effaced, (3) the equilibrium of oil droplet and the "hyaline" cap, or of two oil caps, terminates by the formation of new leading lateral pseudopodium at one side of the middle body region, i.e., a complete new axis is established, however it is not opposite but perpendicular to the original polarity line.

It is a different question why the intracellular streaming continues flowing in the same direction as long as the oil drop remains attached and incorporated into the peripherial cell structure, and why the cell preserves its new polarity after the oil drop is lost. This cannot be explained by the perpetuation of purely mechanical effects only. Such simple explanation is contradicted, for instance, by the anteriority effect, the fact that the second oil droplet injected is handicaped. It is possible that an injected oil drop continues to exert some specific (but not mechanical) influence on the cell motory system, but at present the author cannot propose any plausible mechanism of such permanent effect of oil cap. Anyhow, such direct influence of oil cap, even if it really exists, cannot be the only factor deciding about the behaviour

of amoebae after oil injections. The anteriority effect observed in the double injection experiments, and the fact that amoeba may preserve for long time the polarization induced by the oil cap, even after this cap has been lost, prove that the injected oil drops dominate and rearrange the cell own system of selfregulation of the motory activity.

Presumably the cytoplasmic stream, once initiated, stabilizes its own new direction. Such a positive feedback mechanism is postulated in author's another paper (Grębecka 1977), as resulting from the presence of a relaxing substance emanating from the nucleus, and distributed through the cell interior by the cytoplasmic stream. According to this idea, a new pseudopodium may continuously extend and advance, when the cytoplasmic stream feeds it with the endoplasm which flew around the nucleus and is therefore enriched in the relaxing factor. This may explain why the cytoplasm streaming steadily sustains its direction toward the oil cap, and moreover, the significant fact that the new cell polarity is still kept unchanged after the oil drop has been repulsed to the tail and eventually lost.

At least three further arguments may be found, in favour of this hypothesis, in the experiments with oil drops injected into polypodial amoebae: (1) inversion of the cytoplasm streaming is the first event which precedes the macromorphological reshaping of amoeba and the reorientation of its axis of locomotion, what supports the view that the stream carries the factor which regulates the contraction-relaxation cycle [3], (2) the external morphology of the new front is reshaped earlier than that of the new tail, what speaks in favour of the relaxatory functions of the agent carried by the cytoplasmic stream, and (3) the first oil droplet always dominates over a second equivalent droplet injected later, what proves that it is not the question of simple competition between the direct effects of both oil caps, but that the earlier one had enough time to re-organize and subordinate the cell own mechanism of selfregulation.

The role of cell nucleus in maintaining and stabilizing the cell polarity in amoeba seems to explain particularly well the behaviour of monopodial specimens with artificial oil cap equivalent to the "hyaline" cap, and of polypodial amoebae with two equilibrated oil caps, which have tendency to produce a lateral pseudopodium extending from the middle body region, perpendicularly to the former axis. In such elongat-

[3] This argumentation is related to the concept proposed recently by Grębecki 1976 and 1977, that the cytoplasm circulation should not be considered as direct causal factor of locomotion, the motive force being developed by the semi-rigid cell frame which is not involved itself in the circulation of the internal granular cytoplasm layers.

ed forms two opposite extension gradients are established, and a steady zone should be expected halfway between both extending poles, ie., at the middle body region. The nucleus is in most cases located just in this zone, which ensures its central and relatively stable position in the cell interior. Basing on the hypothesis of a relaxatory agent emanating from the nucleus, we could expect in fact a third relaxation centre to be formed close to the nucleus. This may explain the tendency to produce a lateral pseudopodium in the central zone. With the appropriate content of the factor released from the nucleus, the adjacent region of cortex starts relaxation, lateral pseudopodium is formed and filled with endoplasm flowing from the perinuclear zone. The stream carries more and more of relaxatory factor towards the breach, and in this way it reinforces and stabilizes the new flow direction. The scheme illustrating this hypothetical sequence of events is presented in the Fig. 1.

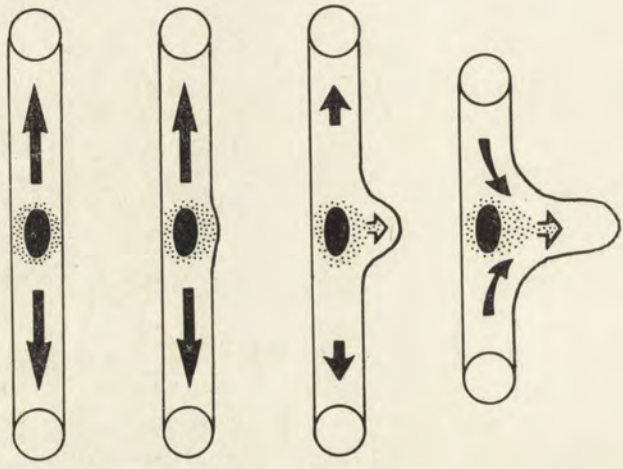

Fig. 1. Scheme explaining the postulated role of cell nucleus and cytoplasnic stream in the formation of lateral pseudopodium by an amoeba distended between two equilibrated oil caps

It should be concluded in general, that the motory polarization of *A. proteus* results from variable contraction-relaxation relationships, which are arranged in the cell in a definite pattern and maintained in order by the action unit composed of cell nucleus and cytoplasnic stream. Direction of the polarization axis may be changed by stimulus strong enough to alter so drastically the formerly established contraction-relaxation gradient, that the nucleus — cytoplasmic stream system must conform to the new pattern and undertake the task of its perpetuation.

RÉSUMÉ

Une gouttelette de l'huile de paraffine injectée à travers le cytoplasme d'une amibe polypodiale, vers la face intérieure de sa membrane, provoque la formation et l'extension d'un grand psuedopode principal terminé par une calotte d'huile, ce qui mène à une réorganisation de la polarité de la cellule. Une telle injection effectuée dans la région d'uroïde réverse entièrement l'axe motorique. Chez les amibes monopodiales on obtient des formes différentes de concurrence entre leur calotte naturelle de "hyaline" et la calotte d'huile injectée, cependant on n'arrive jamais à un réversement complet et définitif de la polarité. L'injection succéssive de deux gouttelettes d'huile équilibrées, dans une amibe polypodiale, mène à la dominance de la calotte qui a été formée la première. Les amibes à deux calottes équilibrées peuvent récuperer leur forme polypodiale en produisant un pseudopode latéral dans la partie centrale de leur corps. On peut supposer que l'injection de l'huile, dans la prémière phase, provoque une extension des couches périfériques de la cellule, et dirige le courant cytoplasmique vers la brèche ainsi formée. Probablement, le courant transporte un facteur régulateur d'origine nucléaire qui provoque la rélaxation des structures corticales, ce qui renforce et stabilise la nouvelle direction prise par le courant cytoplasmique, et installe en fin de compte un nouveau gradient de contraction et de rélaxation dans la cellule, c'est-à-dire établit un nouvel axe de locomotion.

REFERENCES

Allen R. D. 1961: Ameboid movement. In: The Cell. Vol. II. Academic Press. 135–216.

Allen R. D. 1973: Biophysical aspects of pseudopodium formation and retraction. In: The Biology of *Amoeba*. Academic Press, 201–247.

Czarska L. and Grębecki A. 1966: Membrane folding and plasma-membrane ratio in the movement and shape transformation in *Amoeba proteus*. Acta Protozool., 4, 201–239.

Dawson J. A. and Belkin M. 1928: The digestion of oils by *Amoeba dubia*. Proc. Soc. Exp. Biol. Med. 25, 790–793.

Dawson J. A. and Belkin M. 1929: The digestion of oils by *Amoeba proteus*. Biol. Bull., 56, 80–86.

Goldacre R. J. 1956: The regulation of movement and polar organisation in amoeba by intracellular feedback. Proc. 1st Int. Congr. Cybernetics, 715–725.

Goldacre R. J. 1961: The role of cell membrane in the locomotion of amoebae, and the source of the motive force and its control by feedback. Expl. Cell Res., (Suppl.), 8, 1–16.

Goldacre R. J. and Lorch I. J. 1950: Folding and unfolding of protein molecules in relation to the cytoplasmic streaming, amoeboid movement and osmotic work. Nature, 166, 487–499.

Grębecka L. 1977: Behaviour of anucleate anterior and posterior fragments of *Amoeba proteus*. Acta Protozool., 16, 87–105.

Grębecka L. and Grębecki A. 1975: Morphometric study of moving *Amoeba proteus*. Acta Protozool., 14, 337–361.

Grębecki A. 1976: Co-axial motion of the semi-rigid cell frame in *Amoeba proteus*. Acta Protozool., 15, 221–248.

Grębecki A. 1977: Non-axial cell frame movements and the locomotion of *Amoeba proteus*. Acta Protozool., 16, 53–85.

Korohoda W. and Stockem W. 1975: On the nature of hyaline zones in the cytoplasm of *Amoeba proteus*. Microscop. Acta, 77, 129–141.

Marsland D. 1933: The site of narcosis in a cell; the action of a series of paraffin oils on *Amoeba dubia*. J. Cell. Comp. Physiol., 4, 9–33.
Mast S. O. 1926: Structure, movement, locomotion and stimulation in *Amoeba*. J. Morphol., 41, 347–425.
Stockem W., Haberey M. und Wohlfarth-Bottermann K. E. 1969: Pinocytose und Bewegung von Amöben. V Mitteilung. Konturveränderungen und Faltungsgrad der Zelloberfläche von *Amöeba proteus*. Cytobiologie, 1, 37–57.

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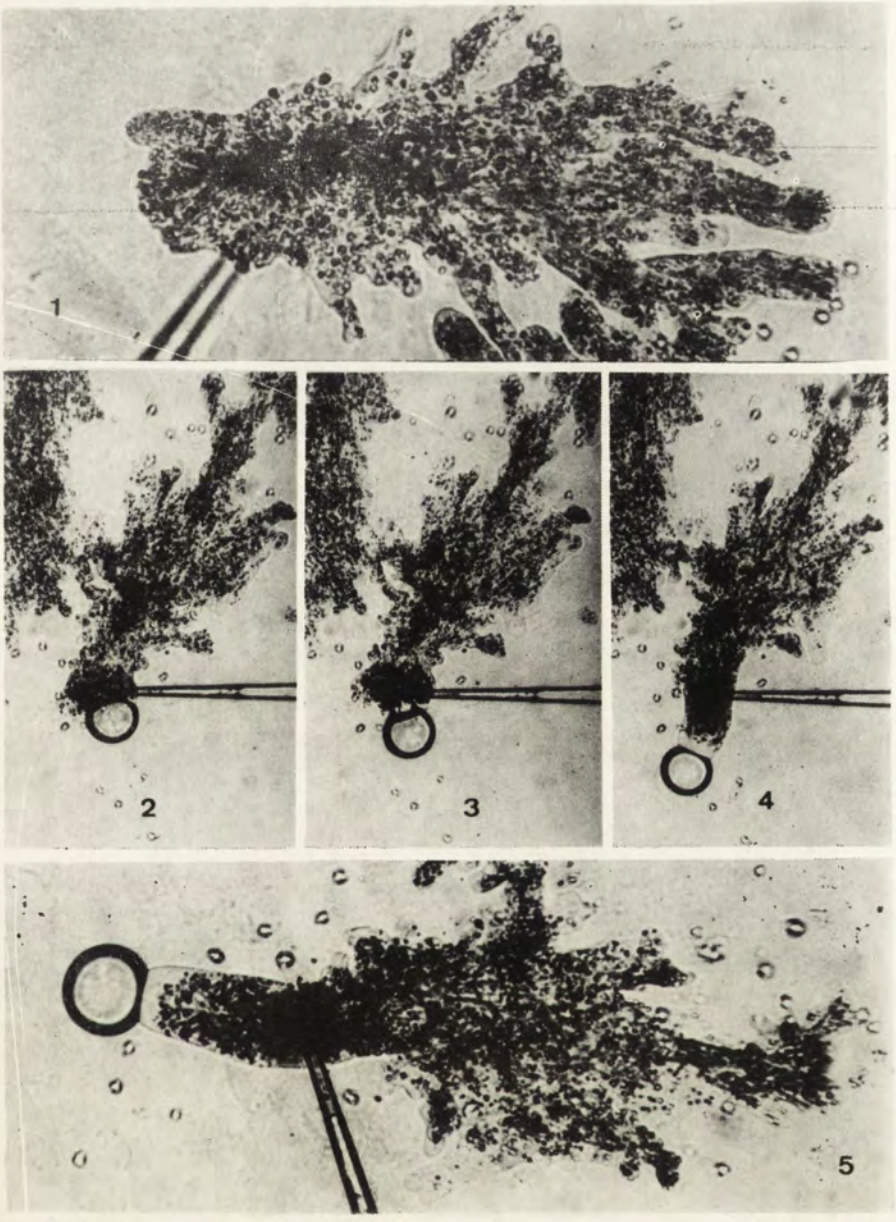
EXPLANATION OF PLATES I–X

1–5: Immediate effects of injection of a paraffin oil drop into the uroid of polypodial amoeba. Time interval between the first and the last picture equal to 10–12 s
6: Time-exposure record of behaviour of polypodial amoeba with oil cap freshly formed at the place of its former uroid. Exposure started 10 s after the injection
7–8: Extension of a huge leading pseudopodium with oil cap, by a polypodial amoeba 8 s after the injection of oil, and maintaining of the induced polarization 20 min later, in spite of loosing the oil droplet
9–10: Polarization of anucleate fragment by the injected oil during the 2nd min after the operation, and rounding up of the fragment 10 min later
11–17: Injection of oil droplet into the posterior region of monopodial amoeba. Injected volume insufficient to disturb continuously the original polarity. The oil cap is active for initial 15 s
18–28: Massive injection of oil which dominates the influence of the "hyaline" cap of a monopodial amoeba, but terminates by effacement of morphological and motory polarization (rounding up), instead of inversing the original polarity
29–30: Formation of lateral pseudopodium in two monopodial specimens with oil drops balancing the influence of their "hyaline" caps
31: Formation of lateral pseudopodium in a specimen, formerly polypodial, after its distension by two equilibrated oil caps
32–41: Recovery of polypodial shape by amoeba which has been distended between two equivalent oil droplets
42–52: Distension of amoeba by two oil droplets almost equal in size but injected at the 10 s interval one after another
53–62: Effect of two oil drops slightly different in size, injected immediately one after another. The amoeba finally rounded up with both drops well attached
63–66: Extension of lateral pseudopodium and recovery, in originally polypodial amoeba with three attached oil drops
67: Final stage of the recovery — a polypodial amoeba still dragging two former oil caps behind its uroid
68: Amoeba distended between two equivalent oil caps with clearly seen steady zone in the middle body region



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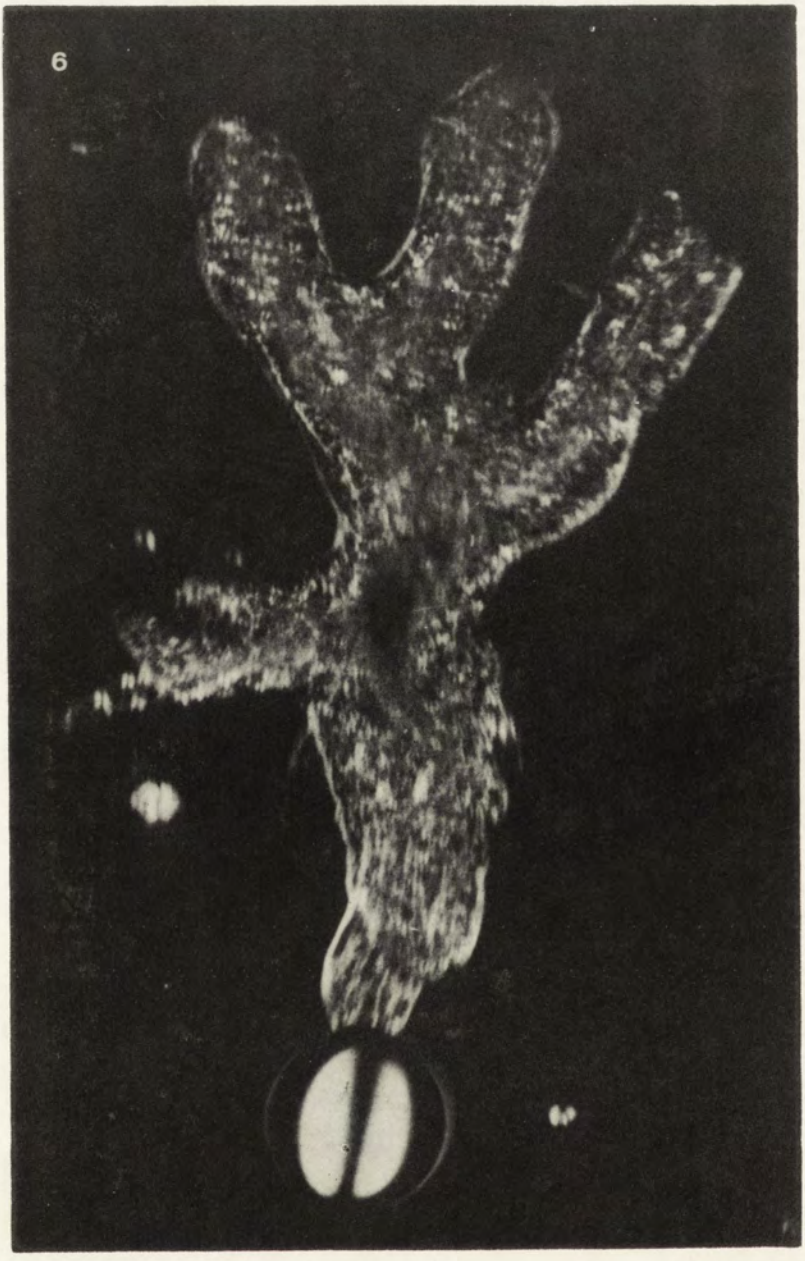

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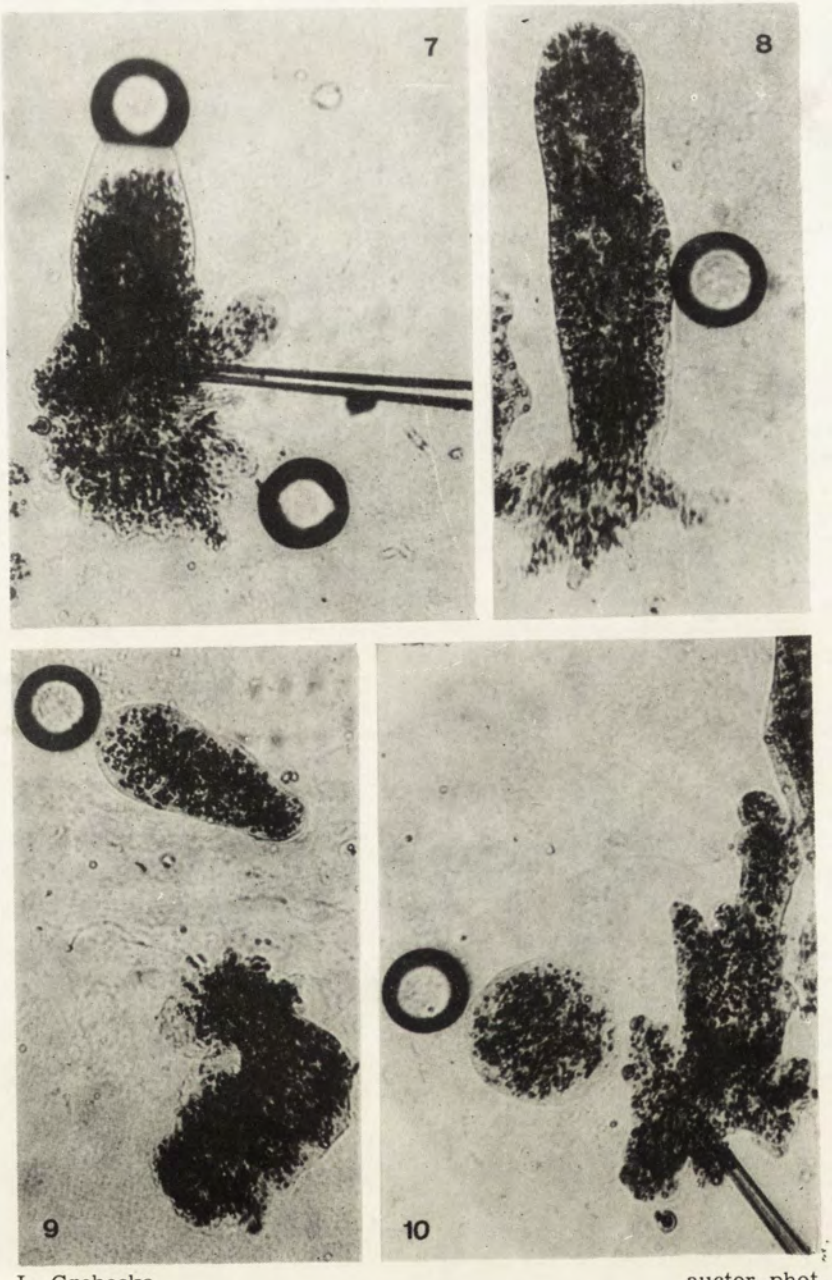

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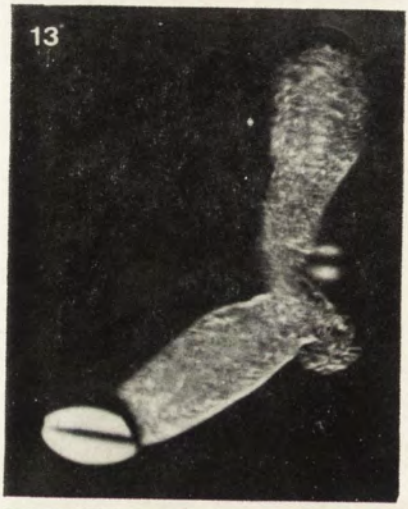

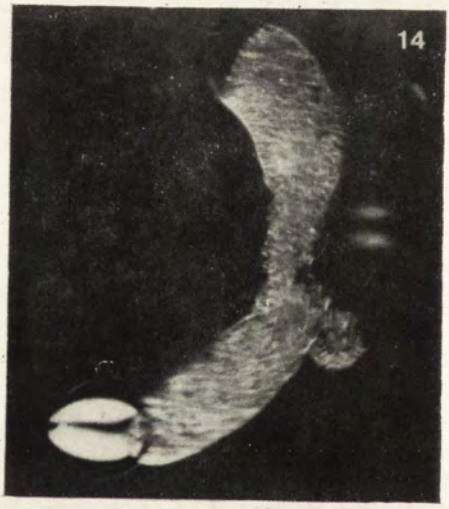

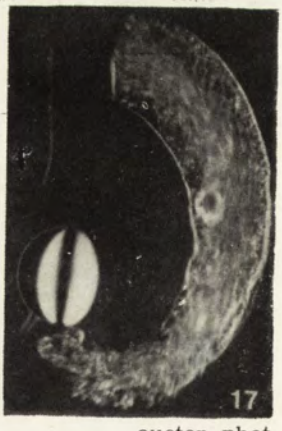

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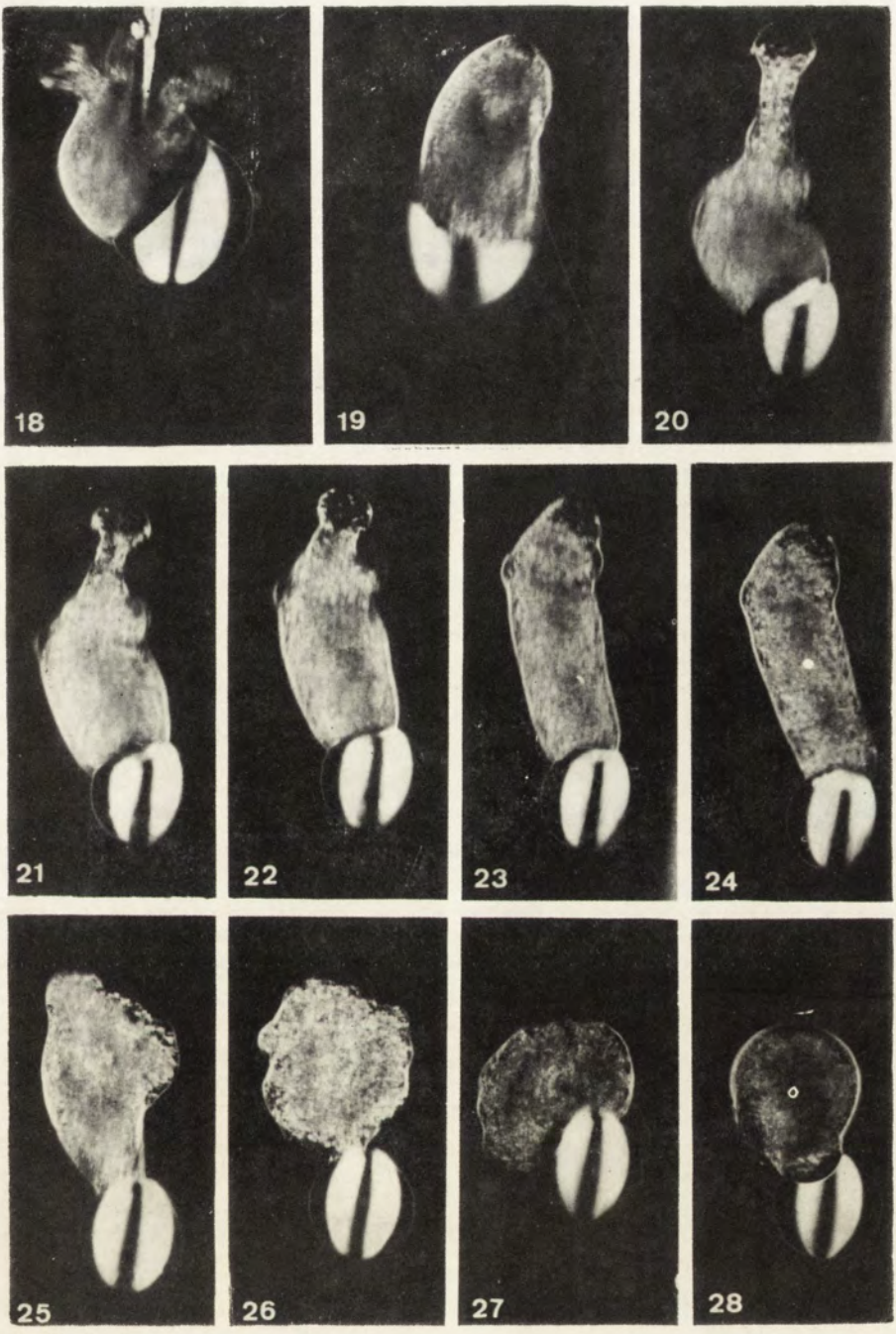

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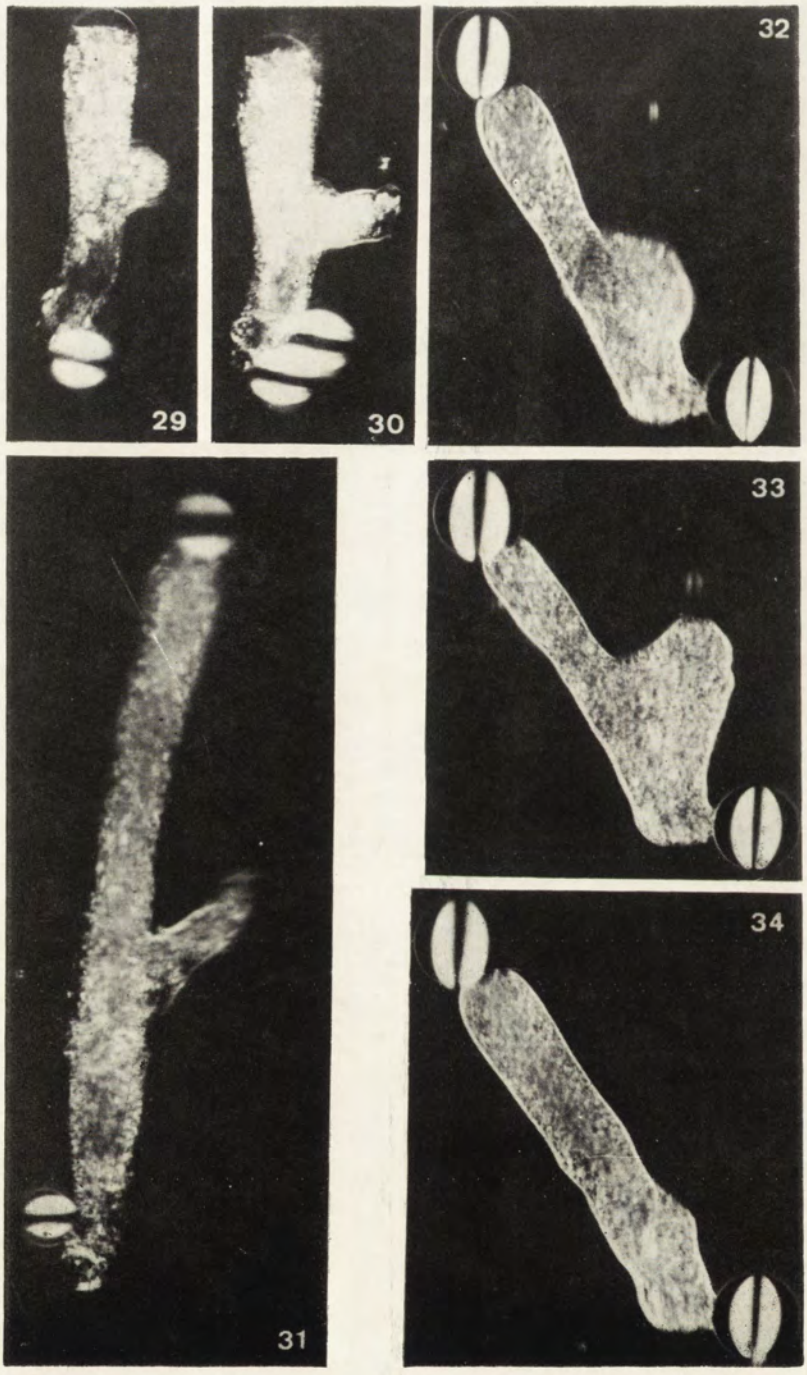

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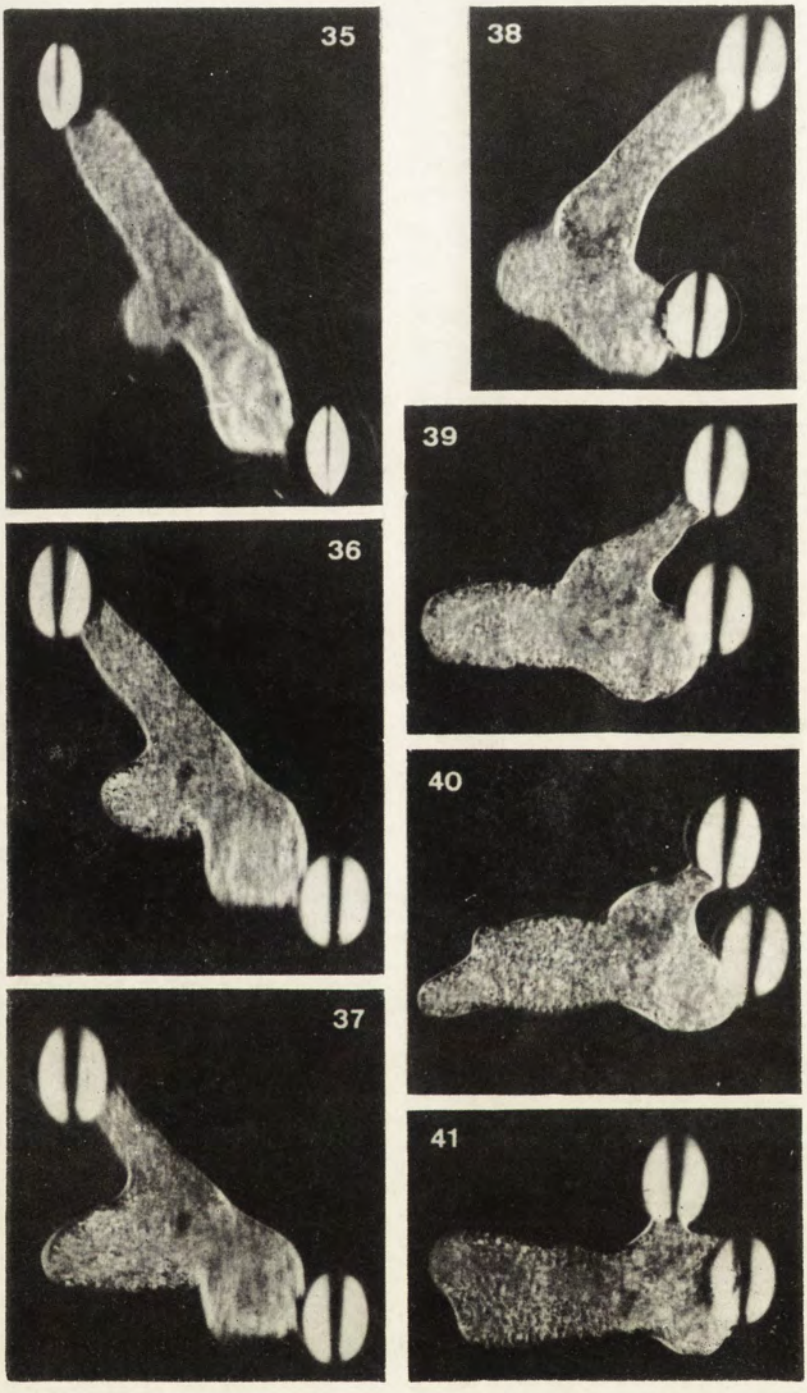

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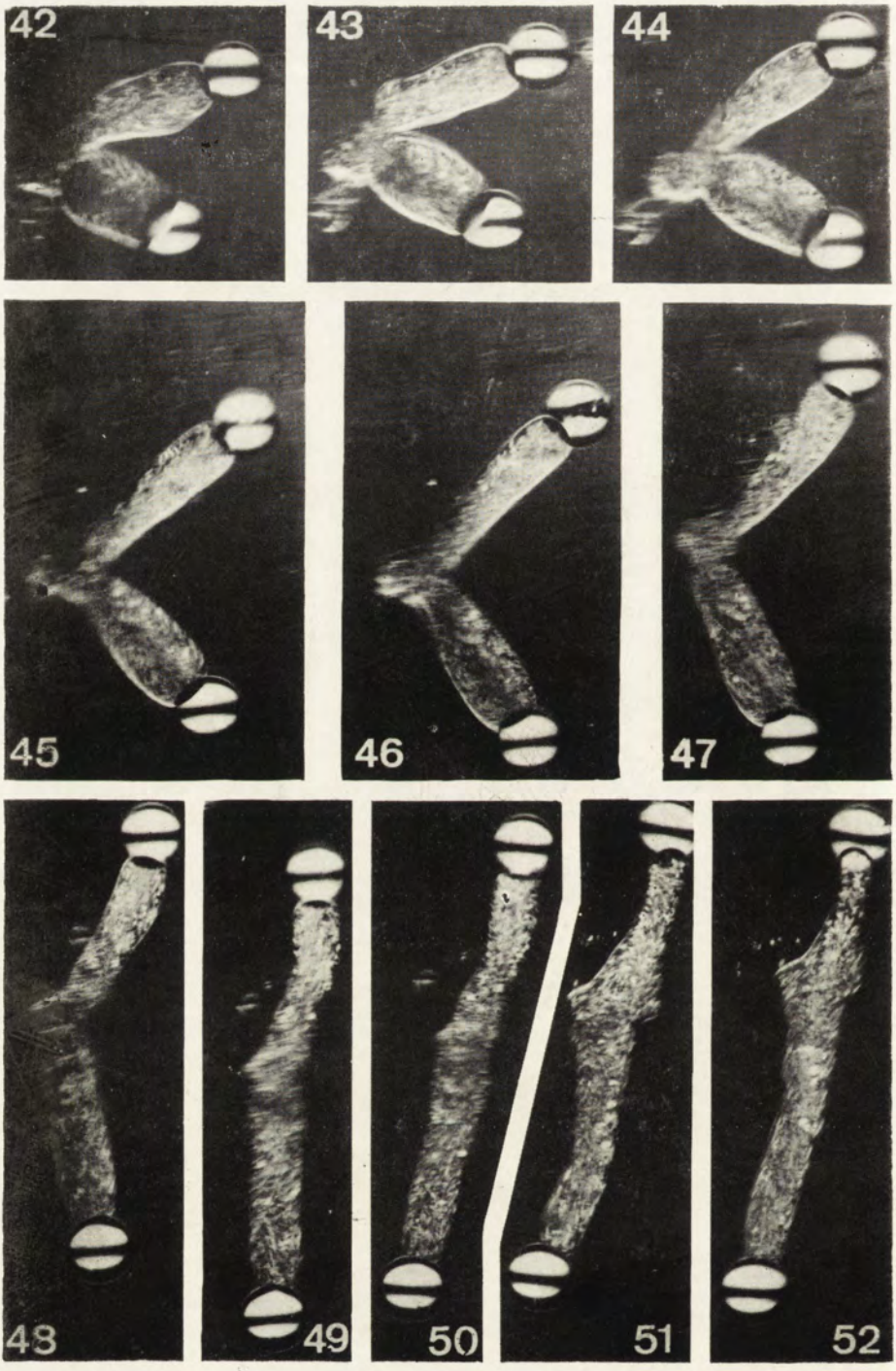

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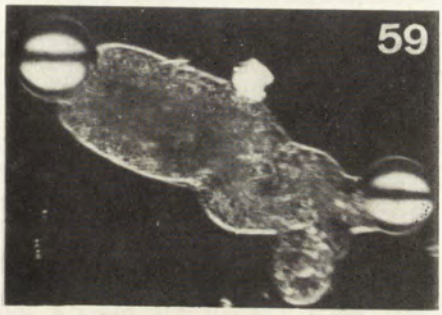

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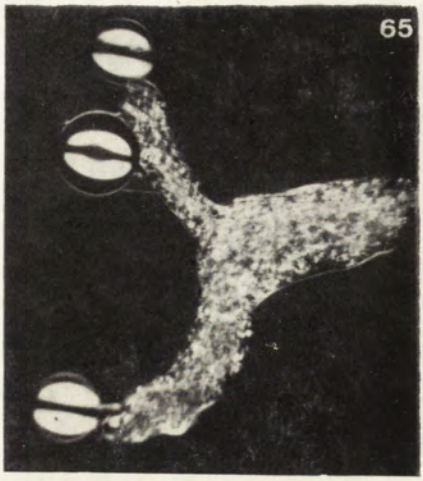

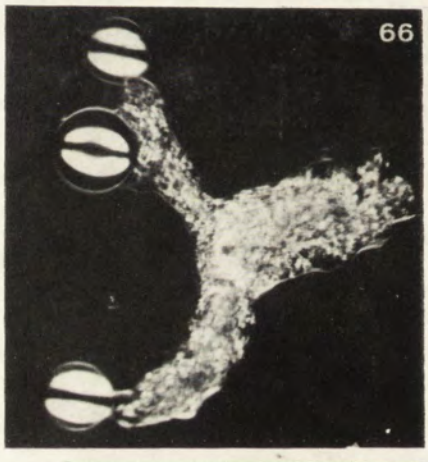

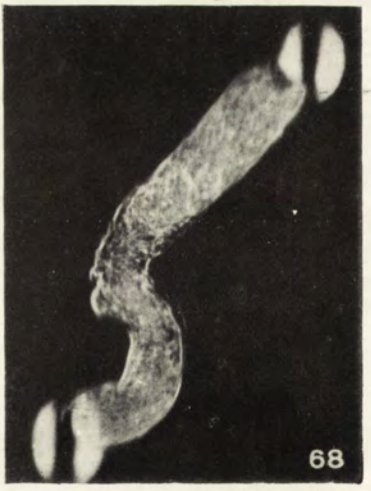

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BOOKS RECEIVED

BIOLOGIE DES *OPHRYOGLENINA*

(Ciliés Hyménostomes Histophages)

L'«Epoque des Ultrastructures» de la Ciliatologie pourra-t-elle aboutir à une Systénatique phylogénétique ou s'agit-il d'une flatteuse illusion?

Mario F. CANELLA et Ilda ROCCHI - CANELLA (Université de Ferrara) 133 figures, 510 pages. Universita degli Studi di Ferrara, 1976. Annali dell'Universita di Ferrara, Sezione III — Biologia animale, suppl. II al vol. III.

TABLE DES MATIÈRES:

Troisième Partie — Connaissances et méprises, anciennes et récentes, sur *Ichthyophthirius multifillis* Fouquet, 1876. I. Préliminaires et historique. II. Morphologie, morphogenèse et biologie d'*Ichthyophthirius*. 1. Matériels et microtechniques, 2. Anatomie générale du théronte, 3. Les structures buccales rudimentaires de type ophryoglénien. 4. Les crêtes ectoplasmiques intercinétiennes et le perforatorium. 5. Comportement du théronte parasitant l'hote, 6. La ciliature somatique et la «raclette» du trophonte, 7. Les appareils osmo-régulateur et nucléaire, et le chondriome, 8. Morphogenèse et morphologie de l'appareil buccal pseudo-trichostomien, 9. Durée de la phase trophique, enkystement et palintomie, 10. La stomatogenèse ophryoglénienne avortée, 11. *Addendum* — Sur une recherche expérimentale de Lom et Cerkasová. III. Problèmes et comparaisons. 1. Absence de processus meïotico-caryogamiques, 2. Les variations du cinétome d'*Ich.* et *Ophryoglena*, 3. Les Ophryoglènes parasites ou présumées telles, 4. Le problème de la spéciation d'*Ichthyophthirius* et quelques comparaison avec d'autres Ciliés dimorphes ou polymorphes, 5. Ophryoglène et Colpodides. IV. Les quiproquos de Roque et de Puytorac 1. Quiproquo numéro un: thérontes d'*Ich.* pris pours des trophontes, 2. Quiproquo numéro deux: théro-trophontes d'*Ich.* pris pour une espèce d'un genre nouveau.

Appendice (M. F. C.). I. La «structural-conservatism hypothesis» de Lynn. II. L'«évolution» des Ciliés peut-elle être éclairée par l'étude des ultrastructures du cortex buccal ou bien par des recherches de biochimie comparative et de génétique moléculaire? III. Un problème embrouillé par surcharge de spéculations: les origines des Ciliés astomes. *Addendum* — Dernières nouvelles taxonomico-phylogénétiques.
Bibliographie, Appendice bibliographique. Table alphabétique des Auteurs.

AN ILLUSTRATED LABORATORY MANUAL OF PARASITOLOGY

Raymond M. CABLE
Fifth Edition, Burgess Publishing Company, 275 pp.

TABLE OF CONTENTS:

Preface to the fifth edition
General References

I. INTRODUCTION: Zoological classification and nomenclature, Parasitism in the animal kingdom

II. PROTOZOA: Introduction to Protozoa. Amebae. Flagellates. Haemoflagellates and related forms. Intestinal flagellates and related forms. Homokaryote ciliates. Heterokaryote ciliates. "*Sporozoa*". Gregarines. Coccidians. Malarial parasites. Other haemosporidians. Piroplasms. Myxosporidians. Microsporidians.

III. HELMINTHS: Mesozoans. Flatworms. Turbellarians. Flukes. Digenetic flukes. Aspidobothrian flukes. Monogenetic flukes. Tapeworms. Thorny-headed worms. Nematodes. Hairworms of "horsehair" snakes. Leeches.

IV. ARTHROPODS: Introduction to arthropods. Mosquitoes. Sand flies or moth flies. Biting midges, mimis, or no-see-ums. Black-flies or buffalo gnats. Horse flies and deer flies. Circle-seamed flies. Syrphid or drone flies. Muscoid flies. Louse flies or tick flies. Bot flies. Fleas. True bugs. Chewing lice. Sucking lice. Parasitic crustaceans. Class Arachnidea. Ticks and mites. Tongue worms.

V. LABORATORY METHODS: Measurement with the microscope. Drawing to scale. Post-mortem examination of vertebrates for parasites. Examination of invertebrates for parasites. *Supra-vitam* staining. Laboratory diagnosis of human parasite infections. Preparation of "permanent" slides

APPENDIX: Reagents and solutions

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In preparation:

J. J. Lipa, K. P. Carl and E. W. Valentine: *Blastocrithidia caliroae* sp. n., a Flagellate Parasite of *Caliroa cerasi* (L.) (*Hymenoptera: Tenthredinidae*) and Notes on its Epizootics in Host Field Populations — J. J. Lipa: *Nosema porphyriniae* sp. n., a New Microsporidian Parasite of *Porphyrinia amasina* Eversman (*Lepidoptera, Noctuidae*) — J. J. Lipa: *Nosema pyrrhocoridis* sp. n. a New Microsporidian Parasite of Red Soldier Bug (*Pyrrhocoris apterus* L.) (*Heteroptera, Pyrrhocoridae*) — J. J. Lipa: Microsporidian Infections of *Galleria mellonella* (L.) (*Lepidoptera, Galleridae*) with the Description of a New Species *Nosema galleria* sp. n. — J. J. Lipa: *Thelohania ostriniae* sp. n., a New Microsporidian Parasite of the European Corn Borer *Ostrinia nubilalis* Hbn. (*Lepidoptera, Pyralidae*) — A. Czapik et A. Jordan: Deux ciliés psammophiles nouveaux: *Hippocomos loricatus* gen. n., sp. n. et *Pleuronema tardum* sp. n. — A. Czapik et A. Jordan: Les ciliés psammophiles de la mer Baltique aux environs de Gdańsk. Partie II — J. Tesařik and R. Janisch: The Effect of Merotomy on RNA Distribution in *Paramecium caudatum* — S. Fabczak: Studies on the Electrical Stimulation of Contraction in *Spirostomum*. III. The Effect of Polycations on Cell Membrane Excitability — B. Tołłoczko: Endocytosis in *Paramecium*. III. Effect of Cytochalasin B and Colchicine — M. Brutkowska, A. Kubalski and J. Kurdybacha: The Influence of EGTA/Ca Buffers on Food Vacuole Formation by *Tetrahymena pyriformis* GL — J. J. Lipa: Infection of *Notocellia uddmaniana* L. (*Lepidoptera: Tortricidae*) by the Microsporidian *Nosema carpocapsae* Paillot — J. J. Napolitano, A. V. LaVerde and H. R. Gamble: Cultivation of *Naegleria* Using Alcohol Killed Bacteria

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