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INSTYTUT BIOLOGII DOŚWIADCZALNEJ IM. M. NENCKIEGO
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Новые виды инфузорий мезопсаммона западного побережья Каспийского моря

New species of psammobiotic ciliates of the western coast of the Caspian Sea

На протяжении многих лет в целом ряде стран всесторонне исследуются морские инфузории микробентоса, играющие большую роль в питании бентических животных. Эти исследования позволили существенно обогатить наши знания, как в области систематики, морфологии и цитологии инфузорий, так и области их общей экологии.

Исследование инфузорий морских песчаных грунтов началось в конце двадцатых годов (Spiegel 1926, Sauerbrey 1928). Подробное описание этой фауны дано в работах Каля (Kahl 1930—1935, 1933); позже появилась целая серия работ о морских инфузориях, населяющих песчаные грунты Северной и Экваториальной Атлантики, Средиземного моря и Японского моря (Fauré-Fremiet 1950, 1951, Bock 1952 a, b, 1953, Fjeld 1955, Nobili 1957, Dragesco 1960, 1963 a, b, 1965, Raikov 1960, 1962, 1963, Vacelet 1961, Borror 1962, 1963, Lepşi 1962, Petran 1963). В результате исследований общее число видов инфузорий, отмеченных в мезопсаммоне, приближается к 400. Однако, несмотря на своеобразные гидрологические и гидрохимические особенности Каспийского моря, вопрос о его фауне инфузорий до настоящего времени оставался неизученным. Некоторые, весьма краткие, сведения об этом встречаются лишь в работах О. А. Гримма (1876), которые сильно устарели. В течение последних 90 лет по инфузориям Каспийского моря никаких исследований не проводилось.

При изучении видового состава и экологии псаммофильных инфузорий азербайджанского побережья Каспийского моря нами были найдены некоторые новые виды, относящиеся к семействам *Enchelyidae, Trachelocercidae, Loxodidae* и *Euplotidae*. Их описание дается ниже, на основании прижизненных наблюдений, а также по тотальным препаратам.

Работа проводилась в мае—августе 1964—1965 гг. на западном побережье Каспия. Материал был обработан в лаборатории цитологии одноклеточных

организмов Института цитологии АН СССР под руководством проф. Ю. И. Полянского.

Пользуюсь случаем выразить глубокую благодарность старшему научному сотруднику И. Б. Райкову за постоянную помощь в работе.

Методика

Исследование проводилось теми же методами, которые использовались в работах Форе-Фремье (Fauré-Fremiet, 1950, 1951), Дражеско (Dragesco 1960) и Райкова (1962, 1963). Пробы песка брали путем соскабливания поверхностного слоя грунта толщиной около 1 см с глубины до 1—1.2 м., чаще 20—40 см от уровня воды. Собранные пробы помещали в кристаллизаторы. Небольшие порции песка с морской водой, взятые из проб, помещались в чашку Петри и энергично встряхивались. Весьма полезным оказалось добавление на чашку Петри 1 мл 12% раствора хлористого магния, под действием которого уменьшался тигмотаксис инфузорий. Определение инфузорий производилось, как правило, на живом материале. Однако описание ниже указанных видов в основном даётся на основании фиксированного материала, импрегнированного серебром по методике Шаттона и Львова (Chatton et Lwoff, 1930). Использование этого метода дало нам весьма ценные сведения о внешней структуре этих инфузорий.

Для изготовления тотальных препаратов по методу Шаттона и Львова инфузории фиксировались в смеси Шампи (5 минут). Инфузории после фиксатора Шампи обрабатывались жидкостью Да-Фано (морская вода 90 мл, 40% формалин 10 мл и $Co(NO_3)_2$ 1 г), которая сменялась 2—3 раза, от двух часов до одних суток (можно хранить и дольше). Инфузории затем отмывались дистиллированной водой (2—3 смены по 10 минут). После этого инфузорий заключали в тонкий слой подогретого 10% желатина, содержащего 0.05% NaCl, который застывал после помещения препарата в холодильник во влажную камеру на 5—10 минут. Затем препарат опускали в холодный 3% раствор $AgNO_3$ на 25—28 минут, тщательно промывали холодной дистиллированной водой (3 смены по 5 минут) и на белом фоне, в холодной дистиллированной воде ставили под кварцевую лампу на 25 минут. После этого препарат обезвоживали спиртами и заключали в бальзам.

Описание новых видов

Lacrymaria monilata sp. n. (Рис. 1, Табл. I 1)

Форма, найденная в Бильгинском районе Каспийского моря (северный берег Апшеронского полуострова) при солености воды 12.55‰, описывается на основании серебренных материалов.

Тело веретенообразное, слегка уплощенное дорсо-вентрально, спереди переходит в короткую шейку и кончается хоботком (рис. 1 А). Ротовые трихиты у фиксированных особей не выражены. Задний конец тела заострен, но не образует вытянутого хвоста. Имеется одна простая сократительная вакуоль на заднем конце (рис. 1 А).

Ресничный покров густой, равномерный, покрывает все тело инфузорий и состоит из 28—33 продольных рядов, образующих слабо выраженную спираль на переднем конце тела.

Макронуклеус всегда представляет собой ясно выраженную цепочку из 5—6

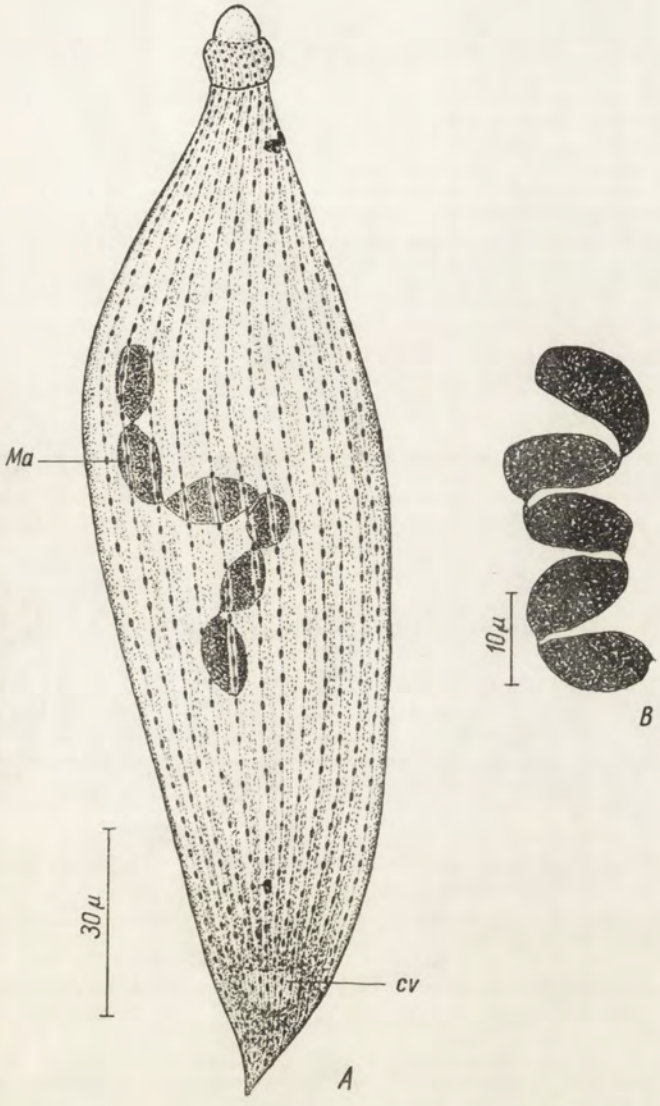


Рис. 1. *Lacrymaria monilata* sp. n. A — общий вид (тотальный препарат, серебрение), B — макронуклеус. *Ma* — макронуклеус, *cv* — сократительная вакуоль

Fig. 1. *Lacrymaria monilata* sp. n. A — general view, whole preparation, silver impregnation, B — macronucleus. *Ma* — macronucleus, *cv* — contractile vacuole

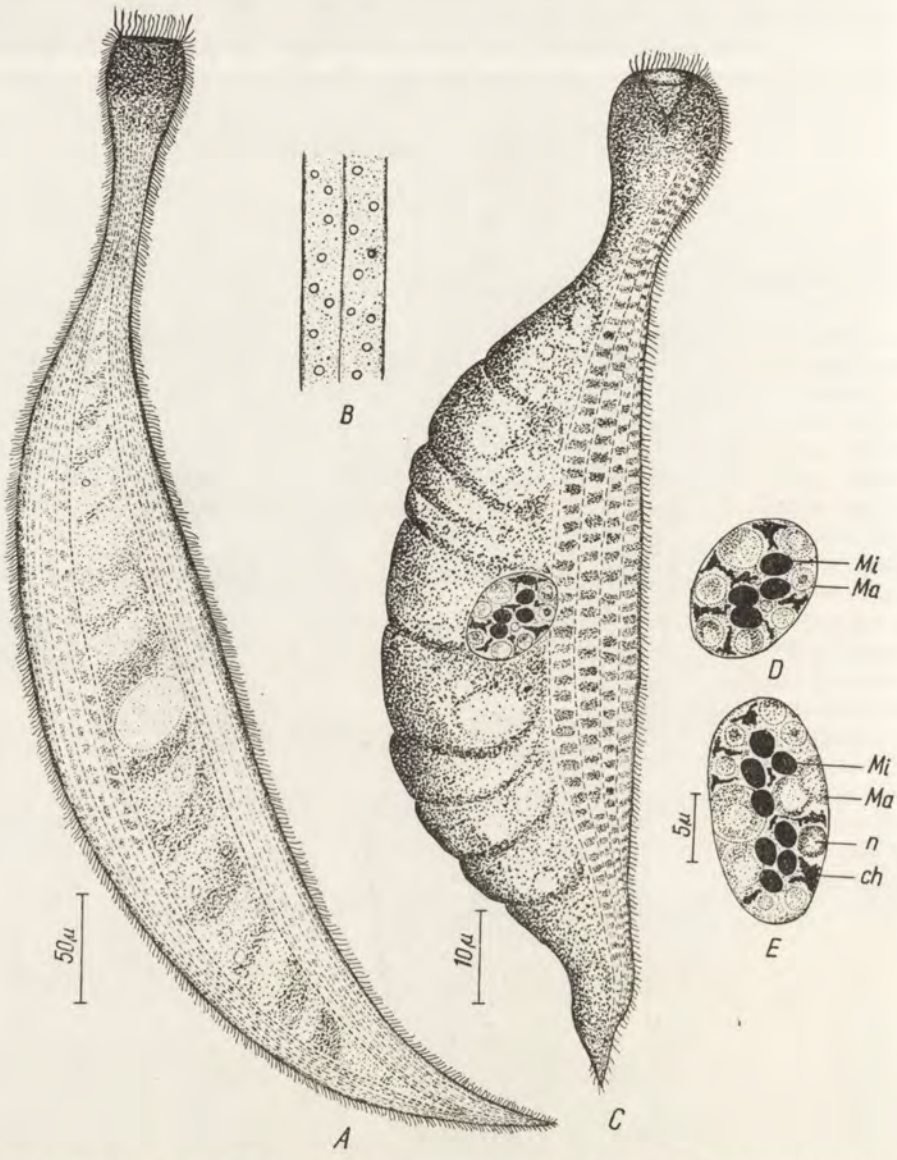


Рис. 2. *Tracheloraphis sarmaticus* Agamaliev et Kovaleva, sp. n. A — общий вид (прижизненно), B — протрихоцисты между рядами ресничек (прижизненно), C — фиксированная особь, D, E — ядра (гемалаун), *Ma* — макронуклеус, *Mi* — микронуклеус, *n* — нуклеола, *ch* — хроматин

Fig. 2. *Tracheloraphis sarmaticus* Agamaliev et Kovaleva, sp. n. A — general view (living material), B — protrichocysts between ciliary rows (living material), C — fixed individual, D, E — nuclei (haemalaun). *Ma* — macronucleus, *Mi* — micronucleus, *n* — nucleolus, *ch* — chromatin

узелков и расположен по продольной оси тела (рис. 1 A, B). Общая длина цепочки составляет 70—80 μ. Эндоплазма содержит разнообразные включения.

Длина тела у фиксированных особей 180 μ; прижизненно — не более 300—400 μ.

Биотоп: мелкий песок с сапробностью (содержанием органического вещества) 0.65% и содержанием карбонатов ($CaCO_3$) 32.11%.

Наша форма отличается от других видов *Lacrymaria* главным образом по строению ядерного аппарата, а от *Lacrymaria balechi* Dragesco иной формой и размерами тела и меньшим числом узелков макронуклеуса.

Tracheloraphis sarmaticus Agamaliev et Kovaleva, sp. n. (Рис. 2)

Этот вид был обнаружен на Апшеронском полуострове и в районе устья р. Куры на западном Каспии. Кроме Каспийского моря, эта форма была найдена В. Г. Ковалевой на Крымском побережье Черного моря. По внешним признакам и по строению ядерного аппарата формы Каспийского и Черного морей очень сходны. Однако при более детальном сравнении выяснилось, что черноморская форма обычно имеет слегка больше ресничных рядов (16—17) и более мелкие микронуклеосы, чем каспийская форма, имеющая обычно 14 рядов. Но поскольку оба признака варьируют и обнаруживают трансгрессию, это не является существенным видовым различием, и поэтому обе формы следует отнести к одному виду.

Форма тела веретенообразная, уплощенная дорсо-вентрально (рис. 2 A). Живые инфузории в падающем свете бесцветные. Цитоплазма забита зернистыми включениями. Передний конец тела образует расширенную головку и заполнен светопреломляющими минеральными гранулами. Терминальный рот, окруженный венчиком длинных ресничек, имеет вид простой воронки без трихоцист и трихитов. Задний конец тела вытянут и кончается иногда загнутым на сторону хвостом. Сократительная вакуоль отсутствует.

Ресничный покров состоит из 13—17 ресничных рядов (чаще всего из 14). Голая спинная полоска, характерная для рода *Tracheloraphis*, довольно широкая и равна примерно 6 ресничным рядам. На живых экземплярах в промежутках между ресничными меридианами хорошо видны протрихоцисты (рис. 2 B). При фиксации пелликула становится поперечно складчатой (рис. 2 C). Питается в основном диатомовыми водорослями, которые хорошо видны в цитоплазме окрашенных экземпляров.

Длина тела 400—600 μ (в вытянутом состоянии).

Ядерный аппарат состоит всегда из одного расположенного в центре тела сложного ядра, внутри которого, как правило, имеется 4 микронуклеуса (рис. 2 D). Такой ядерный аппарат имеется примерно у $^2/_3$ всех особей. У остальной трети экземпляров наблюдается большее число микронуклеусов. Так, из 17 особей с увеличенным числом Mi — 2 экземпляра имели 5 Mi, 6 экземпляров имели 6 Mi, 2 экземпляра — 7 Mi и 7 экземпляров — 8 Mi. Макронуклеусы содержат нуклеолы (*n*) и хромативные зерна (*ch*). По строению ядра инфузория напоминает *Tr. phoenicopterus* (Cohn), у которой обычно имеется 6 микронуклеусов (Райков 1960, 1962).

Tr. sarmaticus отличается от других близких видов того же рода, имеющих одно сложное ядро, следующими признаками: от *Tr. phoenicopterus* (Cohn), переописанной Райковым (1962), и от *Tr. drachi* Dragesco — меньшими размерами тела, меньшим числом ресничных рядов, иным строением ядерного аппарата и другой формой головки. От *Tr. prenanti* Dragesco, *Tr. remanei* Dragesco

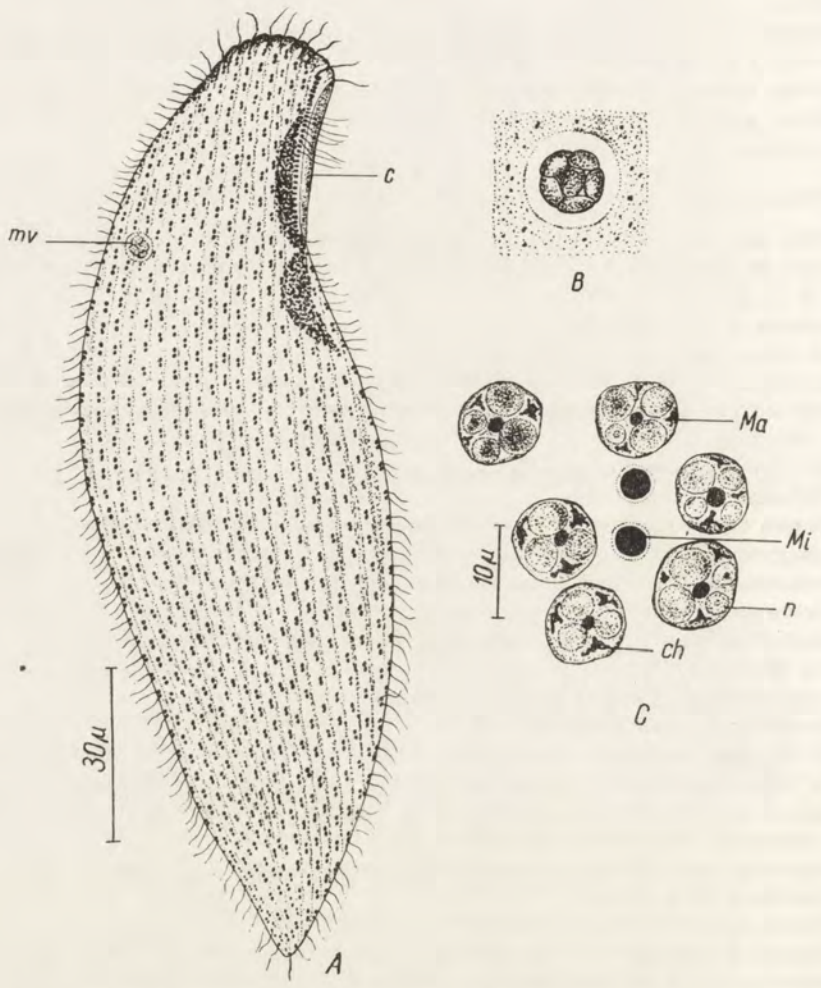


Рис. 3. *Remanella dragescoi* sp. n. A — общий вид (тотальный препарат, серебрение), B — мюллеровская вакуоль с конкрецией, состоящей из нескольких минеральных гранул, C — ядерная группа, состоящая из 6 макронуклеусов и 2 микронуклеусов (Унна), *Ma* — макронуклеус, *Mi* — микронуклеус, *n* — нуклеола, *ch* — хроматин, *c* — рот, *mv* — мюллеровская вакуоль

Fig. 3. *Remanella dragescoi* sp. n. A — general view (whole preparation, silver impregnation), B — Müller's vacuole with concretion consisting of several mineral granules, C — nuclear group consisting of 6 macronuclei and 2 micronuclei (Unna). *Ma* — macronucleus, *Mi* — micronucleus, *n* — nucleolus, *ch* — chromatin, *c* — mouth, *mv* — Müller's vacuole

и от *Tr. incaudatus* (Kahl) — отличается меньшими размерами и формой тела, наличием четырех и более, а не двух микронуклеусов; от *Tr. swedmarki* Dragesco, *Tr. gracilis* Dragesco — другой формой ядерного аппарата и отсутствием околоротовых протрихоцист, имеющихся у *Tr. swedmarki*.

Remanella dragescoi sp. n. (Рис. 3, Табл. I 2)

В настоящее время известно свыше 15 видов *Remanella*, которые все являются типичными представителями интерстициальной фауны (Kahl 1930—1935, 1933, Fauré-Fremiet 1950, 1951, Dragesco 1960, 1965).

При исследовании инфузорий мезопсаммона нами была найдена новая форма, относящаяся к роду *Remanella*. По форме тела и размерам этот вид ближе всего к *Remanella rugosa* Kahl и *R. unicorpusculata* Dragesco.

Форма тела ланцетовидная, сильно сплющенная латерально. Правая (физиологически брюшная) сторона плоская, а левая (физиологически спинная) сторона слегка выпуклая (рис. 3 A). Передний конец тела загнут в сторону брюшного края в виде клюва. Задний конец заострен, имеет форму хвоста, но на серебренных препаратах хвост плохо выражен.

Ресничный покров односторонний, что характерно для рода *Remanella*. Реснички покрывают только правую сторону тела, образуя 20 продольных рядов. Рот в виде продольной щели расположен на правой стороне тела, вблизи брюшного края (рис. 3 A). На правом и заднем крае рта находится скопление зернистого коричневого пигмента. Цитоплазма прозрачная. Вблизи спинного края имеется всегда одна мюллеровская вакуоль со сложной конкрецией. Последняя слагается из нескольких минеральных гранул (рис. 3 B), как у *Remanella rugosa* и *R. unicorpusculata*. Сократительная вакуоль отсутствует.

Ядерный аппарат представлен одной ядерной группой, расположенной компактно в центре тела и состоящей из 6 шаровидных макронуклеусов и 2 микронуклеусов (рис. 3 C). Внутри макронуклеусов хорошо видны нуклеолы и хроматиновые гранулы.

Длина тела у фиксированных особей 150—160 μ; у живых не более 200—250 μ. Питается в основном диатомовыми водорослями.

Биотоп: мелкий гомогенный песок пляжей Шихово и Бильгя (район Апшеронского полуострова) с сапробностью (органическое вещество) 0.52—0.65‰ и содержанием карбонатов ($CaCO_3$) в Шиховском районе — 13.66‰, в Бильгинском — 32.11‰.

Главной отличительной чертой этого вида является форма ядерного аппарата и наличие всего одного мюллеровского тельца.

Euplotes apsheronicus sp. n. (Рис. 4)

Форма тела продолговатая, закругленная на концах. Правый край тела плоский, а левый край слегка выпуклый (рис. 4 A). Выступ на переднем конце (рис. 4 A) является, вероятно, артефактом фиксации. Цитоплазма прозрачная, мало гранулированная. Перистом большой, длина его 40 μ. Адоральная зона мембранелл занимает $^3/_5$ длины тела и состоит из 30—35 мембранелл. Брюшная поверхность постоянно имеет 9 фронто-вентральных, 5 трансверсальных и 4 каудальных цирры. Морфология вентрального аргирома — как у *E. eurystomus* и *E. patella*. Имеется 7—8 рядов дорзальных щетинок (рис. 4 B). Дорзального аргирома на серебренных препаратах не обнаружено.

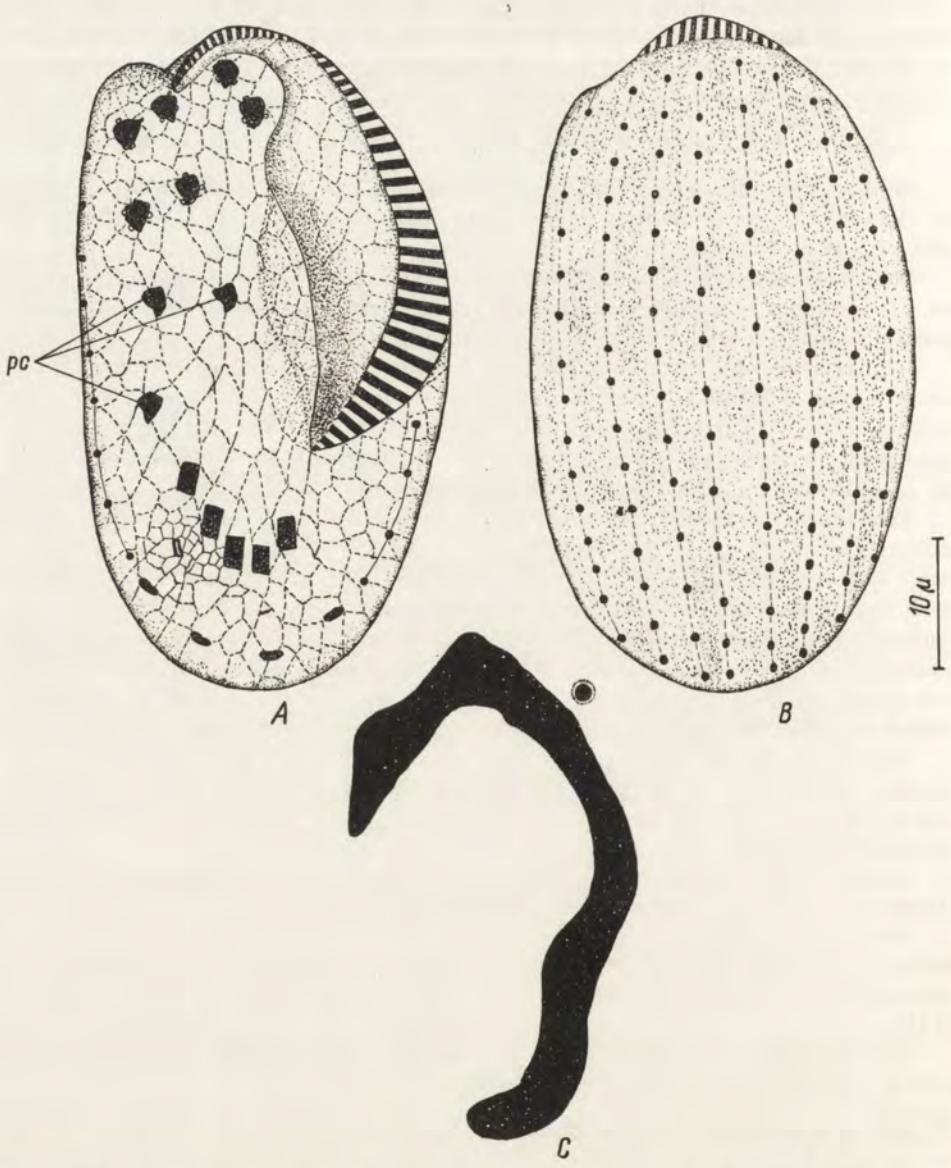


Рис. 4. *Euplotes apsheronicus* sp. n. A — общий вид с брюшной стороны (тотальный препарат, серебрение), B — вид со спинной стороны, C — ядра (реакция Фельгена), *Ma* — макронуклеус, *Mi* — микронуклеус, *pc* — задняя группа фронто-вентральных цирр

Fig. 4. *Euplotes apsheronicus* sp. n. A — general view of the ventral side (whole preparation, silver impregnation), B — view of the dorsal side, C — nuclei (Feulgen reaction), *Ma* — macronucleus, *Mi* — micronucleus, *pc* — posterior group of the fronto-ventral cirri

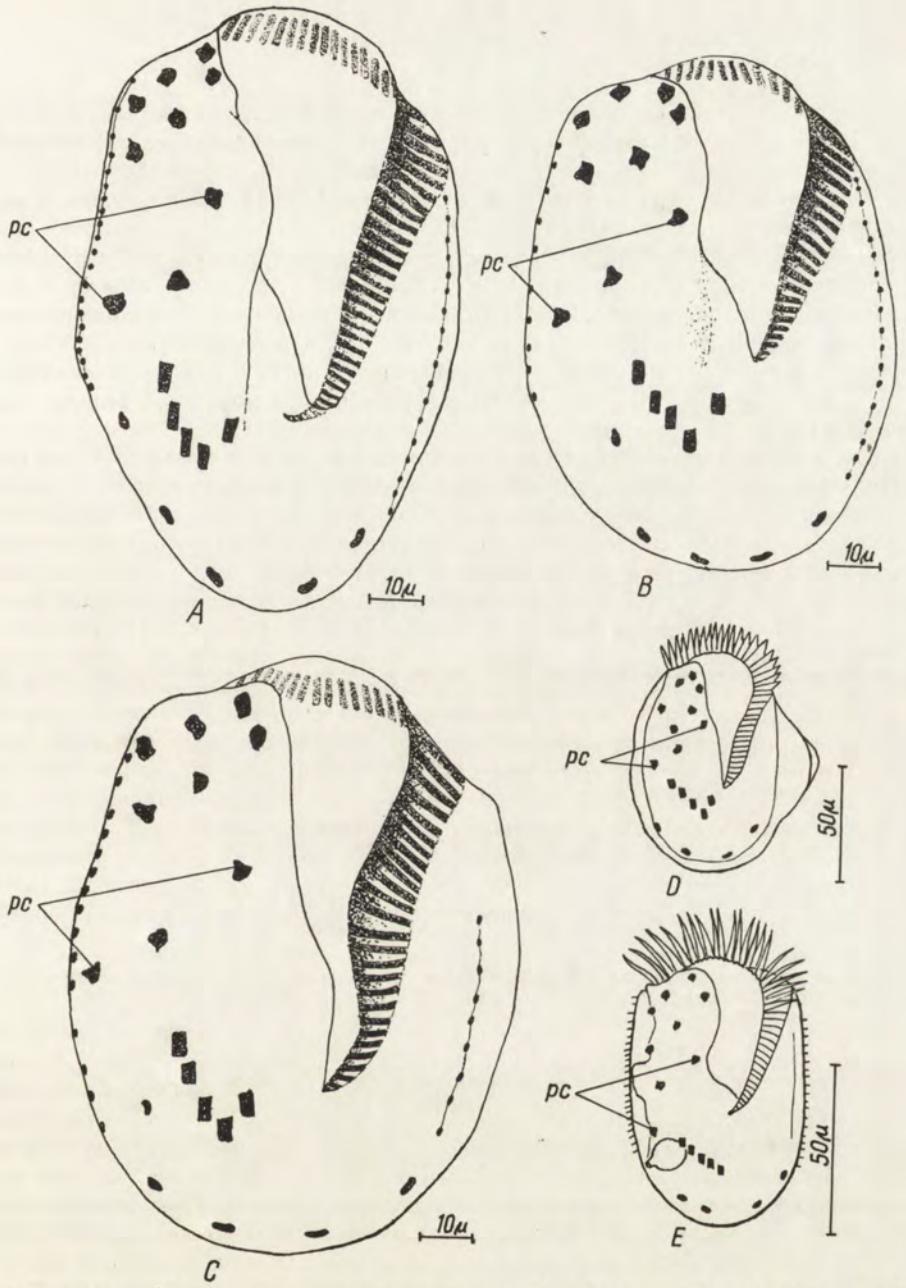


Рис. 5. Схемы брюшной цилиатуры видов, близких к *E. apsheronicus* (вид с брюшной стороны): A — *E. eurystomus* Wrzesniowski, B — *E. patella* Müller, C — *E. plumipes* Stokes, D — *E. leticiensis* Bovee, E — *E. patella* var. *lemani*. A — C — по Tuffrau 1960, D — по Bovee 1957, E — по Dragesco 1960, *pc* — задняя группа фронто-вентральных цирр

Fig. 5. Schemes of the ventral ciliature of species related to *E. apsheronicus* (view of the ventral side): A — *E. eurystomus* Wrześniowski, B — *E. patella* Müller, C — *E. plumipes* Stokes, D — *E. leticiensis* Bovee, E — *E. patella* var. *lemani*. A — C — after Tuffrau 1960, D — after Bovee 1957, E — after Dragesco 1960, *pc* — posterior group of fronto-ventral cirri

Ядерный аппарат по своей форме ближе всего к ядерному аппарату *E. elegans* Kahl. Макронуклеус вакуолизирован, хорошо красится по Фёльгену (рис. 4 С).

Длина тела 50—60 μ; на живом материале не более 70 μ. Обнаружен в мелком песке пляжа Загульба (северный берег Апшеронского полуострова) с соленостью воды 12.55‰. По некоторым признакам этот вид сходен с формами: *E. eurystomus* Wrzesniowski, *E. patella* O. F. Müller, *E. plumipes* Stokes, *E. leticiensis* Bovee, *E. patella* var. *lemani* Dragesco. У всех этих видов фронто-вентральные цирры подразделяются на две группы: передняя группа состоит из 6 цирр, а задняя — из 3 цирр. Расположение передних 6 цирр у всех указанных видов примерно одинаково. Различие касается только 3 задних цирр (рис. 4, 5, *pc*).

Так, у нашего вида (*E. apsheronicus*) эти цирры расположены по углам прямоугольного треугольника, прямой угол которого обращен вперед и вправо (рис. 4, *pc*). У других видов такое расположение задних цирр не встречается. У *E. eurystomus* они расположены по углам тупоугольного треугольника, тупой угол которого обращен назад и влево (рис. 5 А). Кроме того, наша форма отличается от этого вида большим числом рядов дорзальных щетинок, меньшим размером и другой формой макронуклеуса. У *E. patella*, *E. plumipes* и *E. leticiensis* 3 задние цирры расположены приблизительно на одной линии (рис. 5 В, С, D). Наш вид отличается от двух первых видов также своей формой тела и значительно меньшими размерами, а от третьего — главным образом отсутствием крыловидного выступа, который расположен на левом краю тела у *E. leticiensis*. Наконец, у *E. patella* var. *lemani* (рис. 5 Е) цирры расположены по углам тупоугольного треугольника, обращенного вершиной вправо и вверх, что ближе всего к их расположению у *E. apsheronicus*. Однако у *E. patella* var. *lemani* трансверсальные цирры расположены на одной линии, т.е. совершенно иначе, чем у других видов, включая и *E. apsheronicus*. Кроме того, *E. patella* var. *lemani* отличается от *E. apsheronicus* наличием характерного выреза правого края перистома.

Euplotes raikovi sp. n. (Рис. 6, Табл. I—II 3—6)

Форма тела округлая. Отличается тупо закругленным задним и слегка суженным передним концом (рис. 6 А). Цитоплазма прозрачная. Перистом занимает больше половины тела; длина его составляет 35 μ. Адоральная зона мембранелл образует передний край перистомальной полости, спускается по левому краю тела и состоит из 30—34 мембранелл. Вентральная часть тела имеет всего 7 фронто-вентральных, 5 трансверсальных и 2—3 каудальных цирры. Две задние фронто-вентральные цирры расположены близ правого края тела на одной продольной линии. Немного левее от них расположено основание одной щетинки (рис. 6 А). Имеется одна сократительная вакуоль. Вентральный аргиром очень характерен и несколько напоминает соответствующие структуры у *E. minuta* и *E. cristatus*. В результате серебрения на дорзальной поверхности тела обнаруживаются 6—7 рядов дорзальных щетинок (рис. 6 В). Расположенные между ними ячейки аргирома очень своеобразны и по своему типу сходны с таковыми у *E. patella*.

При серебрении нами были обнаружены несколько особей, готовящихся к делению. У них наблюдаются закладки новых цирр будущих дочерних особей, расположенные двумя группами выше и ниже середины тела (рис. 6 С). Адоральная зона мембранелл еще не подвергается изменениям.

Макронуклеус имеет форму перевернутой буквы „С" и сильно вакуолизирован (рис. 6 D). Микронуклеус вплотную прилегает к макронуклеусу.

Длина тела 60 μ. Обнаружен в мелком песке пляжа Шихово (южный берег Апшеронского полуострова) с соленостью воды 12.65‰. Грунт представляет собой серый песок с мелкими ракушками, сапробность которого составляет 0.52% органического вещества, а содержание карбоната ($CaCO_3$) — 13.66%.

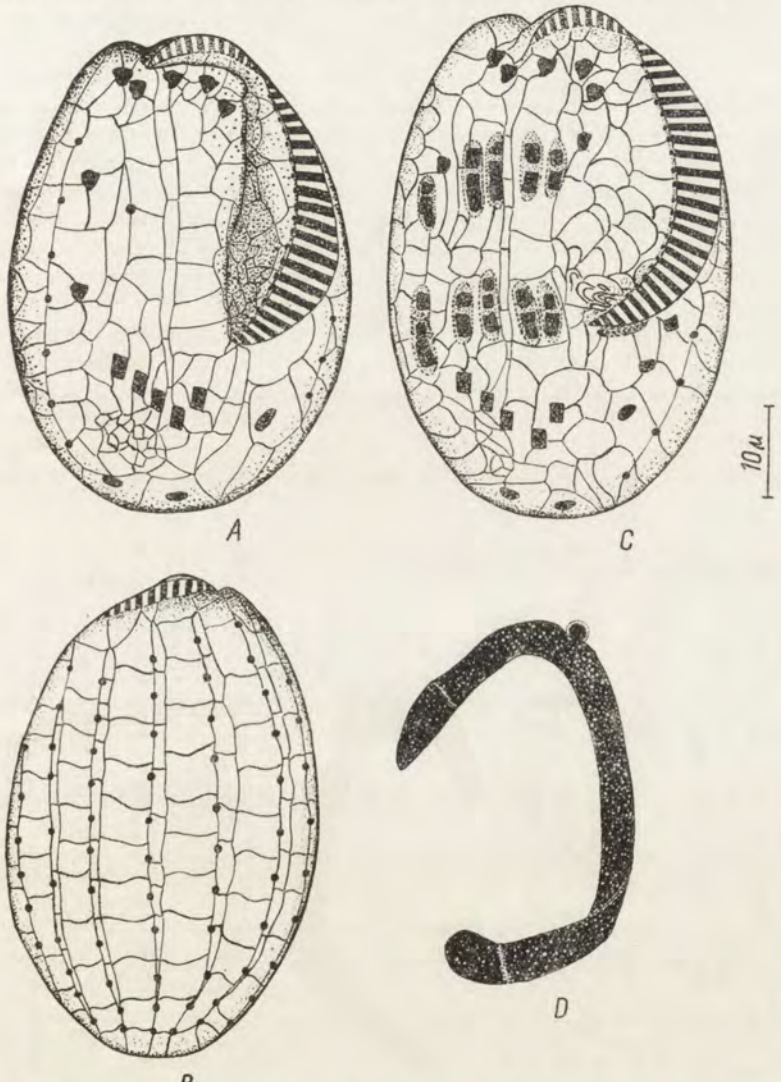


Рис. 6. *Euplotes raikovi* sp. n. A — общий вид с брюшной стороны (тотальный препарат, серебрение), B — вид со спинной стороны, C — особь, готовящаяся к делению (вид с брюшной стороны), D — ядра (реакция Фельгена)

Fig. 6. *Euplotes raikovi* sp. n. A — general view of the ventral side (whole preparation, silver impregnation), B — view of the dorsal side, C — individual prior the division (ventral side view), D — nuclei (Feulgen reaction)

Главными отличительными чертами этого вида является малое количество фронто-вентральных цирр (7) и их характерное расположение.

Euplotes poljanskyi sp. n. (Рис. 7, Табл. II 7—9)

Этот вид встречается на пляже Загульба и в большом количестве в Худатском и устье-Куринском районах побережья Каспийского моря. Форма тела овальная и симметричная по краям (рис. 7 А). Цитоплазма прозрачная с разнообразными включениями. Перистом занимает больше половины тела, длина его 38 μ. Адоральная зона мембранелл состоит из 36—40 мембранелл. На брюшной стороне тела имеется 8 фронто-вентральных, 5 трансверсальных и 3 каудальных цирры. Из фронто-вентральных цирр передние 6 расположены так же, как у *E. apsheronicus*. Имеется одна сократительная вакуоль, расположенная на правом крае трансверсальных цирр. Строение вентрального аргирома сходно с таковым у *E. apsheronicus*. На дорзальной стороне тела обнаруживается 7 рядов дорзальных щетинок. Межщетинковый аргиром чаще всего имеет вид сетки, которая по типу строения напоминает дорзальный аргиром *E. eurystomus*.

Макронуклеус *E. poljanskyi* n. sp. по своей форме сходен с макронуклеусом *E. apsheronicus*. Микронуклеус расположен на некотором расстоянии от макронуклеуса.

Длина тела 55—70 μ. Обнаружены в песках западного побережья Каспия с соленостью воды 11.35—11.75‰. Сапробность (органическое вещество) грунта 0.48—0.77% (устье Куры и Худат) и содержание карбоната ($CaCO_3$) 18.19—19.26%.

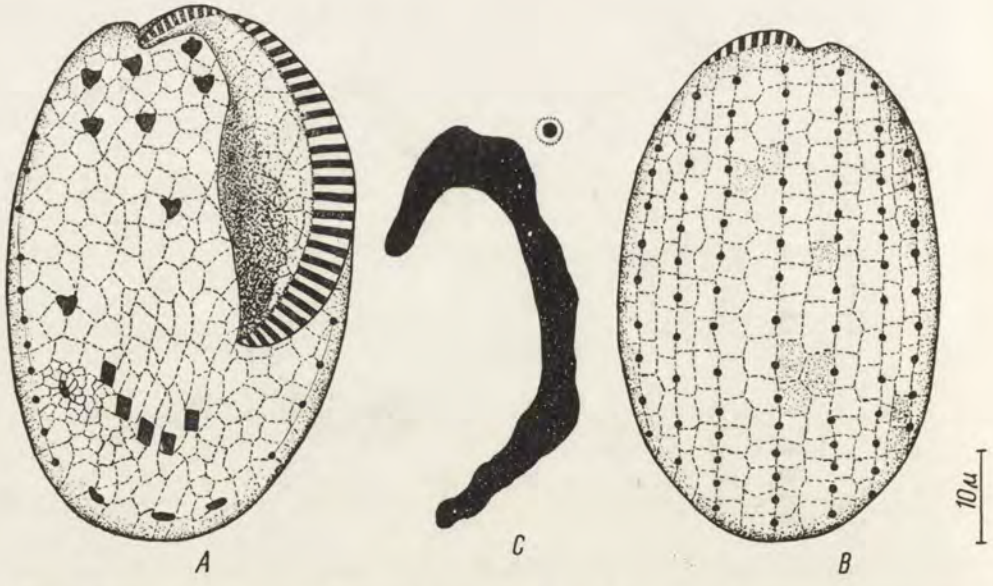


Fig. 7. *Euplotes poljanskyj* sp. n. A — общий вид с брюшной стороны (тотальный препарат, серебрение), B — вид со спинной стороны, C — ядра (гемалаун)

Fig. 7. *Euplotes poljanskyi* sp. n. A — general view of the ventral side (whole preparation, silver impregnation), B — dorsal side view, C — nuclei (haemalaun)

Главной отличительной чертой этого вида является малое количество (всего 8) фронто-вентральных цирр и их расположение. Последнее напоминает таковое у группы видов *E. apsheronicus — E. patella — E. plumipes — E. eurystomus — E. leticiensis* (рис. 4, 5), но отличается исчезновением одной из цирр задней группы фронто-вентральных и одной из каудальных.

Выводы

В мелком песке Азербайджанского побережья Каспийского моря было обнаружено 6 новых видов, относящихся к семействам *Enchelyidae, Trachelocercidae, Loxodidae* и *Euplotidae: Lacrymaria monilata* sp. n., *Tracheloraphis sarmaticus* sp. n., *Remanella dragescoi* sp. n., *Euplotes apsheronicus* sp.n., *Euplotes raikovi* sp.n., *Euplotes poljanskyi* sp. n. Описания этих видов даются на основании прижизненых наблюдений и изучения фиксированного материала, импрегнированного серебром по методике Шаттона и Львова. Среди этих видов *Euplotes poljanskyi* sp. n. встречается в большом количестве.

SUMMARY

Six new infusorian species belonging to the families *Enchelyidae, Trachelocercidae, Loxodidae* and *Euplotidae* have been discovered in the sandy bottom of the western coast of the Caspian sea. Description of these species are founded on study of living ciliates and of fixed material impregnated with silver by the Chatton and Lwoff (1930) method.

1. *Lacrymaria monilata* sp.n. (Fig. 1, Pl. I 1). Body spindle-shaped, 300—400 μ long. Posterior body end sharpened, but without any prolonged "tail". One simple posterior contractile vacuole. The ciliature consists of 28—33 longitudinal rows, assuming a slightly spiral course at the anterior body end only. Macronucleus moniliform, consisting of 5—6 nodes, stretched along the body axis.

2. *Tracheloraphis sarmaticus* Agamaliev et Kovaleva, sp.n. (Fig. 2). Found in the Caspian Sea as well as on the Crimean coast of the Black Sea. The forms from both localites are practically alike. Body form spindle-shaped, length 400 to 600μ. The anterior end forms a thickened "head" filled with refractory granules. Posterior end with tail-like process. The ciliature consists of 13 to 17 kineties (usually of 14). The naked dorsal stripe is wide and equals to 6 kineties or so. The nuclear apparatus consists always of one centrally located "complex nucleus", containing usually 4 micronuclei (Fig. 2 C, D). Animals possessing 5—8 micronuclei are also met with. The nuclear apparatus of this species resembles that of *Tr. phoenicopterus* (Cohn), re-described by R a i k o v (1962), but *Tr. sarmaticus* has a smaller number of kineties.

3. *Remanella dragescoi* sp.n. (Fig. 3, Pl. I 2). Body lancet-like, strongly flattened laterally, 200—250 μ long. Posterior end sharpened and tail-like. The body form and dimensions closely resembling those of *R. rugosa* Kahl and *R. unicorpusculata* Dragesco. Ciliature consisting of 20 longitudinal kineties. A single Müller's vesicle with a compound inclusion body near the dorsal body ridge (Fig. 3 A, B). The nuclear apparatus represented by one compact nuclear group occupying the centre of the body and consisting of 6 spherical macronuclei and 2 micronuclei (Fig. 3 C). The main distinguishing characters of this species are the appearance of its nuclear apparatus and the single Müller's vesicle.

4. *Euplotes apsheronicus* sp.n. (Fig. 4). Body elongate, with rounded ends. The right body margin straight, the left one slightly convex. Body length 60—70μ. Adoral zone as long as 3/5 of the body, consists of 30 to 35 membranellae. Ventral surface constantly has 9 fronto-ventral, 5 transversal and 4 caudal cirri. 7 to 8 rows of dorsal bristles are present (Fig. 4 A, B). The nuclear apparatus resembling that of *E. elegans* (Tuffrau 1960). The differences between *E. apsheronicus* and some related species, i.e. *E. eurystomus* Wrzesniowski, *E. patella* O. F. Müller, *E. plumipes* Stokes, *E. leticiensis* Bovee, *E. patella* var. *lemani* Dragesco (Fig. 5), are stated.

5. *Euplotes raikovi* sp.n. (Fig. 6, Pl. I 3, 4, II 5, 6). Body rounded with broad posterior and slightly narrower anterior end, 60 μ long. The peristome occupies more than a half of the body length (35 μ). The adoral zone consists of 30—34 membranellae. The ventral body side has only 7 fronto-ventral, 5 transversal and 2 or 3 caudal cirri. There are 6—7 rows of dorsal bristles. The pattern of the dorsal argyrome is very characteristic and resembles that of *E. patella*. The macronucleus is C-shaped and strongly vacuolized. The main distinguishing character of this species is the small number and characteristic pattern of the fronto-ventral cirri.

6. *Euplotes poljanskyi* sp.n. Fig. 7, Pl. II 7—9). Body oval, with symmetrical margins, 70μ long. The peristome occupies more than a half of the body length. The adoral zone consists of 36 to 40 membranellae. There are 8 fronto-ventral, 5 transversal, and 3 caudal cirri as well as 7 rows of dorsal bristles. The pattern of the dorsal argyrome resembles that of *E. eurystomus*. The macronucleus resembles that of *E. apsheronicus*.

ЛИТЕРАТУРА

Bock K. J. 1952 a: Über einige holo- und spirotriche Ciliaten aus den marinen Sandgebieten der Kieler Bucht. Zool. Anz. 149, 107—115.

Bock K. J. 1952 b: Zur Ökologie der Ciliaten des marinen Sandgrundes der Kieler Bucht I. Kieler Meeresforsch. 9, 77—89.

Bock K. J. 1953: Zur Ökologie der Ciliaten des marinen Sandgrundes der Kieler Bucht II. Kieler Meeresforsch. 9, 252—256.

Bovee E. C. 1957: *Euplotes leticiensis* n.sp., from the Letician Drainage into the Amazon River. J. Protozool. 4, 124—128.

Borror A. C. 1962: *Euplotes minuta* Yocom (*Ciliophora, Hypotrichida*). J. Protozool. 9, 271—273.

Borror A. C. 1963: Morphology and ecology of the benthic ciliated Protozoa of Alligator Harbor, Florida. Arch. Protistenk. 106, 465—534.

Chatton E. et Lwoff A. 1930: Imprégnation, par diffusion argentique de l'infraciliature des Ciliés marins et d'eau douce, aprés fixation cytologique et sans dessication. C. R. Soc. Biol. Paris, 104, 834—836.

Dragesco J. 1960: Les Ciliés mésopsammiques littoraux (systématique, morphologie, écologie). Trav. Sta, biol. Roscoff, n.ser. 12, 1—356.

Dragesco J. 1963 a: Compléments à la connaissance des Ciliés mesopsammiques de Roscoff. I. Holotriches. Cahiers Biol. Mar. 4, 91—119.

Dragesco J. 1963 b: Compléments à la connaissance des Ciliés mésopsammiques de Roscoff. II. Heterotriches et Hypotriches. Cahiers. Biol. Mar. 4, 251—274.

Dragesco J. 1965: Ciliés mésopsammiques d'Arique noire. Cahiers Biol. Mar. 6, 357—399.

Fauré-Fremiet E. 1950: Ecologie des Ciliés psammophiles littoraux. Bull. biol. France Belg. 84, 35—75.

Fauré-Fremiet F. 1951: The marine sand-dwelling ciliates of Cape Cod shores. Biol. Bull. 100, 59—70.

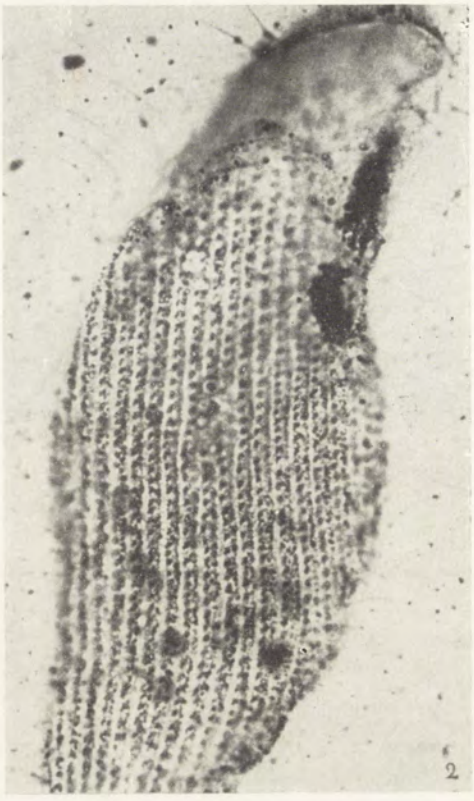
Fjeld P. 1955: On some marine psammobiotic ciliates from Drøbak (Norway). With remarks on a method for quantitative studies of micropsammon. Nytt Mag. Zool. 3, 5—65.
Гримм О. А. 1876: Каспийское море и его фауна. Тр. Арало-Касп. эксп. 2.
Kahl A. 1930—1935: Wimpertiere oder Ciliata. Teile 1—4. Die Tierwelt Deutschlands, 18, 21, 25, 30. G. Fischer, Jena.
Kahl A. 1933: Ciliata libera et ectocommensalia. Tierwelt der Nord-und Ostsee, 23, Teil II, 3, 29—146, Leipzig.
Lepsi I. 1962: Über einige insbesondere psammobionte Ciliaten vom rumänischen Schwarzmeer-Ufer, Zool. Anz. 168, 460—465.
Nobili R. 1957: Contributo all'ecologia dei Ciliati psammofili del Golfo di Napoli. Boll. Zool. 24, 211—225.
Petran A. 1963: Contributii la cunoasterea microfaunei de ciliate psammofile din maren Negra-litoralul Rominesc. Stud. Cercet. Biol., Ser. Biol. anim. 15, 187—197.
(Raikov I. B.) Райков И. Б. 1960: Интерстициальная фауна инфузорий песчаной литорали Дальнезеленецкой бухты (Восточный Мурман). Тр. Мурманск. морск. биол. инст. 172—186.
Raikov I. B. 1962: Les Ciliés mesopsammiques du littoral de la Mer Blanche (URSS), avec une description de quelques espèces nouvelles ou peu connues. Cahiers Biol. Mar. 3, 325—361.
(Raikov I. B.) Райков И. Б. 1963: Инфузорий мезопсаммона Уссурийского залива (Японское море). Зоол. журн. 42, 1753—1766.
Sauerbrey E. 1928: Beobachtungen über einige neue oder wenig bekannte marine Ciliaten. Arch. Protistenk. 62, 355—407.
Spiegel A. 1926: Einige neue marine Ciliaten. Arch. Protistenk. 55, 184—191.
Tuffrau M. 1960: Révision du genre *Euplotes*, fondée sur la comparaison des structures superficielles. Hydrobiologia. 15, 1—77.
Vacelet E. 1961: La faune infusorienne des "sables a Amphioxus" des environs de Marseille. Bull. Inst. océanogr. Monaco, 1202, 1—12.

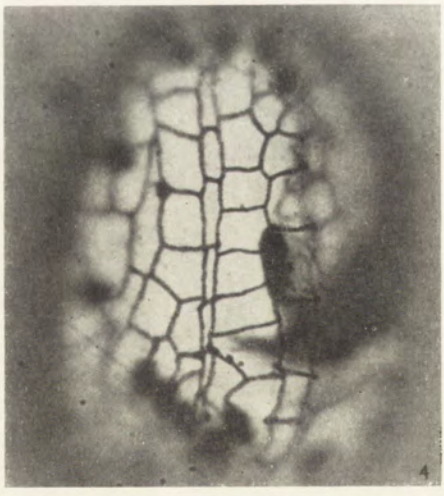
ПОДПИСИ К ТАБЛИЦАМ I — II

1: *Lacrymaria monilata* sp. n., общий вид (серебрение, об. 40, ок. 15)
2: *Remanella dragescoi* sp. n., вид с правой стороны тела (серебрение, об. 90, ок. 10)
3—6: *Euplotes raikovi* sp. n. (серебрение, об. 90, ок. 15): 3 — общий вид с брюшной стороны; 4 — брюшной аргиром; 5 — спинной аргиром; 6 — особь, готовящаяся к делению (вид с брюшной стороны)
7—9: *Euplotes poljanskyi* sp. n. (об. 90, ок. 15): 7 — общий вид с брюшной стороны (серебрение); 8 — спинной аргиром (серебрение); 9 — делящаяся особь (гемалаун)

EXPLANATION OF PLATES I — II

1: *Lacrymaria monilata* sp. n., general view (silver impregnation, 40 × 15)
2: *Remanella dragescoi* sp. n., view of the right body side (silver impregnation, 90 × 10)
3—6: *Euplotes raikovi* sp. n. (silver impregnation, 90 × 15): 3 — general view of the ventral side; 4 — ventral argyrome; 5 — dorsal argyrome; 6 — individual prior the division (view of the ventral side)
7—9: *Eupotes poljanskyi* sp. n. (90×15): 7 — general view of the ventral side (silver impregnation); 8 — dorsal argyrome (silver impregnation); 9 — individual in division (haemalaun)





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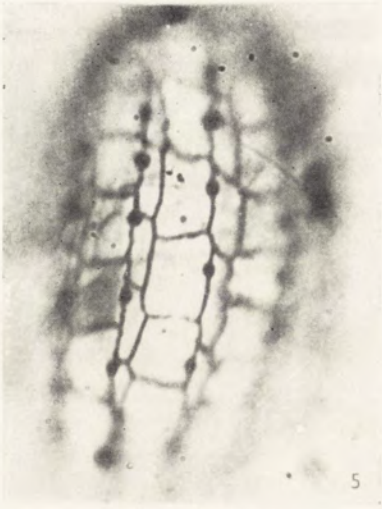

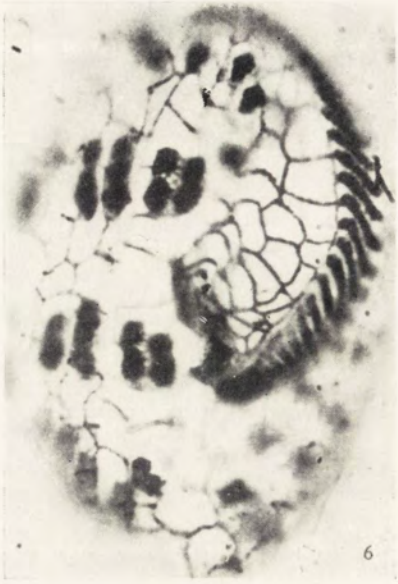

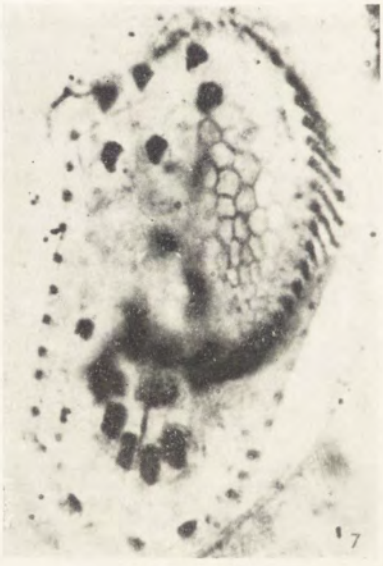

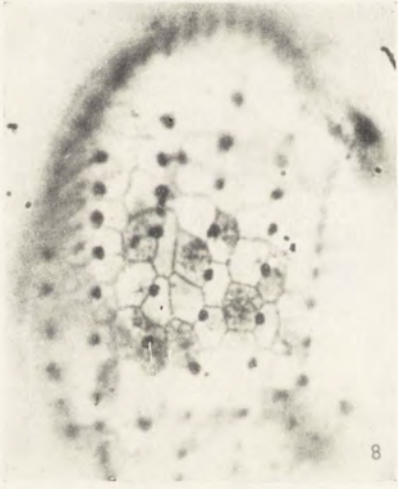

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Studies on *Trichomonas vaginalis* Donné, III.[1] Biometric features of *T. vaginalis* from different clinical forms of trichomoniasis

Studia nad *Trichomonas vaginalis* Donné, III.
Cechy biometryczne *T. vaginalis* w różnych klinicznych postaciach rzęsistkowicy

It may be found in many textbooks of protozoology that the length of *Trichomonas vaginalis* ranges from 10 to 30 μ and its width from 10 to 20 μ (Doflein und Reichenow 1949—1953, Kudo 1954). Jirovec 1960 is of the opinion that the size of trichomonads depends on the clinical picture of infection and distinguishes small, mean, and large forms of the size 8 to 10×4 to 6 μ, 15 to 30 μ, and 30 to 45 μ, respectively. He has reported that small forms are encountered in acute infections while the mean and large ones in chronic disease. Kupferberg (after Okła 1954) paid attention to the changes in the size of cultured *T. vaginalis* and associated them with pH of the medium.

Materials and methods

Because of the lack of detailed data on the variability of the size of *T. vaginalis* attempts were made[2] to elaborate the following biometric features of this protozoon: length, width, surface, volume, length-width index (shape index), and plazma-nucleus index.

The studies comprised three groups of females, 20 to 25 individuals in each group, with symptom-free, acute, and chronic infection; the material was taken during the early estrogenic and late luteal phases of the menstrual cycle. The number of *T. vaginalis* tested in one female ranged from 20 to 200. In direct preparations the biometric features of 1500 protozoa (50 from each of 30 females) from different forms of infection were elaborated; in addition there were measured 200 trichomonads of each of 3 strains cultured for 1 month on the medium of Pawłowa. In stained preparations the following

[1] The first and second part of this work were published elsewhere (see references)

[2] Due to the initiative of the head of the Department docent dr Rościsław Kadłubowski.

numbers of protozoa were tested: (1) 20 from each of 30 females during a mass examination, (2) 50 from each of 15 females from the out-patient gynecological clinic, and (3) 200 from each of 3 patients of the gynecological clinic. No statistically significant differences were found in the values of the biometric features of the protozoa from the same form of infection; thus the results were put together and a total of 1950 trichomonads was estimated in these preparations. Three hundred parasites from the vaginal discharge of 11 females treated with arsenic compounds because of chronic trichomoniasis were examined separately since after treatment the trichomonads differed considerably from the average ones.

The measurements were taken with a Zeiss micrometer. Protozoa in direct preparations of the vaginal discharge or from culture were measured in phase contrast microscope (magnification 400×) after an instant killing with a saturated solution of corrosive sublimate, those in stained preparations of the vaginal discharge after fixation in methyl alcohol and staining with the Giemsa or Wright method (1000× hom. imm).

The longer axis (in direct preparations) or that running through the aggregation of blepharoplasts and the center of the nucleus (in stained preparations) was regarded as the protozoon length, and a perpendicular line running through the middle of the length as its width. The surface of the projection of the protozoa and their nuclei was measured with a polar planimeter after their drawings were made with the aid of a drawing attachment.

The volume of the cell and the nucleus was calculated after Oliva, Part and Barberis according to the formula:

$$V = \frac{\pi}{6} \cdot \frac{(D_1 - D_2)^3}{2}$$

where D_1 and D_2 represent the length and the width of the cell or nucleus, or after Kawiak from the size of protozoon surface according to the formula $V = \sqrt{(p)^3}$, where p = surface.

In the elaboration of biometric features of *T. vaginalis* the following data were calculated: arithmetic mean, standard deviation, standard error, mode, median, amd the coefficients of skewness, variation, and excess (Szulc 1961, Hill 1962). For comparing the results the statistical significance of the differences between corresponding features was calculated. All the data are given in the attached Tables.

The mode to median ratio and the coefficient of skewness proved the asymmetry of the distribution and the direction of this asymmetry (left or right), the coefficient of excess the degree of flattening of the curve of distribution of the feature tested, and the coefficient of variation a smaller or greater aggregation round the mean value.

Results and discussion

Variation in size

The length of *T. vaginalis* in direct preparations of the vaginal discharges from different forms of trichomoniasis ranged from 5.80 to 37.70 μ; the average length of 1500 parasites was 20.57 ± 5.17 μ.

In stained preparations the length ranged from 4.35 to 47.85 μ. The average length of 1950 protozoa was 24.78±5.32 μ. The width calculated from the same material ranged from 4.35 to 39.15 μ, the mean being 20.22±6.81 μ.

Hence the average length and width of *T. vaginalis* in stained preparations are larger than those found in direct preparations. Similarly the trichomonad surface is larger in stained preparations (mean 78.34 μ^2) than in direct ones (mean 61·03 μ^2). This seems to result from flattening of the trichomonad cell during preparation of the smear from the vaginal discharge. On general the cells are usually larger in direct histological preparations, and their smaller size in stained preparations is ascribed to the dehydrating action of reagents used for fixation. It should be assumed that alcohol has a similar effect also in the case of *T. vaginalis* but flattening of protozoa during preparation of the smears is of greater importance.

The mean length-width index was 1.26 in direct preparations and 1.45 in stained ones. The difference is statistically significant ($p < 0.01$). Thus it may be supposed that round forms are more often encountered in direct preparations.

Distinct differences associated with the form of trichomoniasis were encountered in the values of all biometric features of *T. vaginalis*. Detailed data referring to the protozoa from the vaginal discharge of females with symptom-free, acute, and chronic infection are presented in Table 1. The data were obtained from direct preparations.

It results from these data that the mean length of *T. vaginalis* was largest in chronic infection and smallest in acute one; the difference in these values is statistically significant ($p < 0.01$). The mean width of the protozoon is largest in chronic infection while smallest one in symptom-free disease. The difference in these means is statistically significant.

The data from Table 1 show that in all forms of infection the average length of trichomonads is smaller than the mode and median values. This proves an asymmetric distribution of the feature and slightly right asymmetry; individuals longer than the mean dominated among protozoa tested. In chronic infection the distribution of the discussed feature resembles the normal one.

The mean width of *T. vaginalis* is smaller than the mode and median, but the two latter are more similar in all forms of infection. This may prove that the distribution of this feature resembles more the normal one; in addition, in all groups the coefficients of skewness and excess are smaller than those for the length.

The difference in the mean shape indices for asymptomatic and acute cases is statistically significant ($p < 0.01$), that for acute and chronic disease is insignificant.

A plus value of the coefficient of skewness in asymptomatic and acute infection proves a left asymmetric distribution. The distribution of the shape index is most similar to a normal one in acute infection while in chronic infection it shows a right asymmetry (*K* has minus value).

The zone of variation in the shape index is broadest in asymptomatic infection; trichomonads are of different shape but forms similar to round ones dominate slightly. Similarly round forms dominate in acute infection which is proved by a high plus value of the coefficient of skewness and low value of the mean of the shape index. On the other hand in chronic infection oval

Table 1

Biometric features of *T. vaginalis;* direct preparations of the vaginal discharge from females with asymptomatic (A), acute (B), and chronic (C) infection

Feature	Length			Width			Shape index		
From of infection	A	B	C	A	B	C	A	B	C
Zone of variation	5.8— 26.1 μ	8.7— 29.0 μ	8.7— 37,7 μ	2.9— 17.4 μ	5.8— 20.3 μ	5.8— 31.9 μ	0.40— 1.68	0.87— 1.33	0.87— 1.56
Mean	19.2 μ	17.3 μ	23.2 μ	14.8 μ	16.1 μ	20.0 μ	1.38	1.18	1.21
Standard deviation	±5.1 μ	±4.6 μ	±5.6 μ	±6.0 μ	±5.7 μ	±4.9 μ	±0.39	±0.26	±0.41
Standard error	±0.23	±0.21	±0.25	±0.27	±0.26	±0.22	±0.18	±0.12	±0.18
Mode	21.1 μ	19.6 μ	25.1 μ	16.1 μ	18.1 μ	21.2 μ	1.29	1.16	1.22
Median	20.9 μ	17.9 μ	22.7 μ	15.1 μ	18.1 μ	21.9 μ	1.36	1.15	1.20
Coefficient of skewness	−0.77	−0.49	−0.33	−0.21	−0.35	−0.23	+0.23	+0.71	−0.24
Coefficient of excess	+0.68	+0.57	−0.11	+0.11	+0.23	−0.11	−0.27	+0.13	+0.23
Coefficient of variaton	26.7%	26.7%	24.0%	40.7%	35.6%	24.3%	28.3%	22.0%	33.9%

or fusiform protozoa are more often encountered; this is indicated by the *K* value and the mean.

In direct preparations from one-month-old cultures the length of *T. vaginalis* ranged from 5.80 to 25 μ. The mean length for 3 cultured strains obtained from acute, chronic, and asymptomatic infection was 15.28±3.16 μ, 14.98±4.34 μ, and 15.07±3.39 μ, respectively, the differences being statiscally insignificant. The width of the protozoon from these cultures ranged from 2.90 to 13.16 μ. The mean width of the individual strains was 11.64± ±3.13 μ, 12.05±5.11 μ, and 12.11±4.48 μ, respectively, the differences being statistically insignificant. The shape index ranged from 0.8 to 1.68, the means were 1.25±0.40, 1.21±0.33, and 1.20±0.52, respectively, and the differences were statistically insignificant.

Similar variation in *T. vaginalis* size was obtained in studies with stained preparations. The data are given in Table 2. It is shown that the average length of the protozoon was largest in chronic infection and smallest in acute one. The difference in these means is statistically significant ($p < 0.05$) as also in the case with the differences in the means for asymptomatic and acute infection and asymptomatic and chronic one.

The mean width was largest in chronic infection and smallest in symptom-

Table 2

Biometric features of *T. vaginalis*; stained preparations of the vaginal discharge from females with asymptomatic (A), acute (B), and chronic (C) infection

Feature	Length			Width			Shape index		
Form of infection	A	B	C	A	B	C	A	B	C
Zone of variation	4.3 — 47.8 μ	4.3 — 39.1 μ	4.3 — 47.8 μ	4.3 — 36.2 μ	4.3 — 39.1 μ	4.3 — 39.1 μ	1.0 — 3.0	0.6 — 2.8	0.6 — 2.6
Mean	26.0 μ	19.7 μ	23.6 μ	17.9 μ	19.1 μ	23.6 μ	1.86	1.26	1.25
Standard deviation	±7.8 μ	±5.2 μ	±6.0 μ	±7.2 μ	±6.4 μ	±6.4 μ	±0.33	±0.46	±0.33
Standard error	±0.29	±0.23	±0.23	±0.29	±0.29	±0.23	±0.01	±0.02	±0.01
Mode	27.5 μ	18.3 μ	28.0 μ	1 .5 μ	15.5 μ	21.0 μ	1.82	1.19	1.23
Median	26.3 μ	19.2 μ	28.7 μ	17.6 μ	13.4 μ	23.0 μ	1.83	1.19	1.20
Coefficient of skewness	−0.19	−0.28	−0.11	−0.20	−0.56	−0.39	−0.12	−0.24	−0.15
Coefficient of excess	−0.12	−0.15	−0.13	−0.37	−0.50	−0.13	−0.76	−0.39	−0.57
Coefficient of variation	29.8%	26.5%	21.0%	40.4%	33.3%	27.3%	12.4%	36.5%	26.4%

free one. The mean width of the protozoon was similar to its mean length in acute infection. The differences between the mean widths of *T. vaginalis* from different forms of infection were statistically significant ($p < 0.01$).

The mean length of *T. vaginalis* differs only slightly from the mode and median. This together with the low values of the coefficients of skewness and excess proves a small asymmetry of the distribution of this feature; in symptom-free infection there is observed a right asymmetry while in acute and chronic disease a left one.

Diagrams of variation in the length of *T. vaginalis* in asymptomatic, acute, and chronic infection are presented in Fig. 1. The curve allows to state that the smallest trichomonads are encountered in acute infection and that more than 50 per cent of individuals tested in this group is of the length smaller than the mean. The curves are similar in asymptomatic and chronic infection although the difference in corresponding means is statistically significant.

The width of *T. vaginalis* shows a left asymmetrical distribution in all groups, this being most expressed in acute infection. The diagrams of variation in *T. vaginalis* width in different forms of infection are presented in Fig. 2.

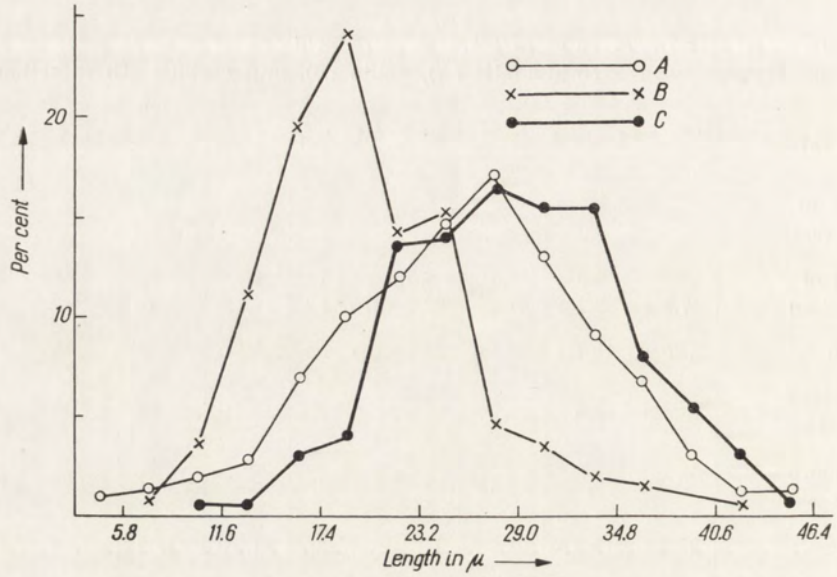


Fig. 1. Variation of length in *T. vaginalis* in asymptomatic (A), acute (B) and chronic (C) infection

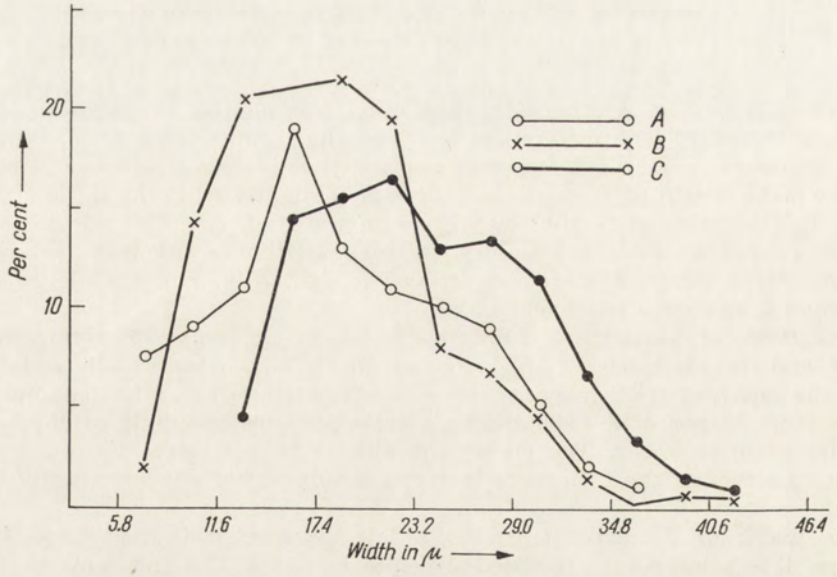


Fig. 2. Variation of width in *T. vaginalis* in asymptomatic (A), acute (B) and chronic (C) infection

Variation in shape

The highest values of the protozoon shape index were obtained in asymptomatic infection; this may prove a repeated (more often than in other forms of infection) oval, fusiform and other similar shape. The shape index in acute and chronic infection ranged within narrower limits.

The difference in the mean value of the shape index between asymptomatic and acute or chronic infections is statistically significant ($p < 0.01$), while that between acute and chronic cases in insignificant.

A low plus value of the coefficient of skewness in asymptomatic infection indicates the presence of a small left asymmetry while a high plus value of the coefficient of excess indicates an aggregation round the mean. In acute infection high minus value of the coefficient of excess indicates a flattening and the coefficient of skewness an asymmetric distribution (left asymmetry). In chronic infection the values of the mean, mode and median are more similar; high value of the coefficient of kurtosis proves an aggregation of the

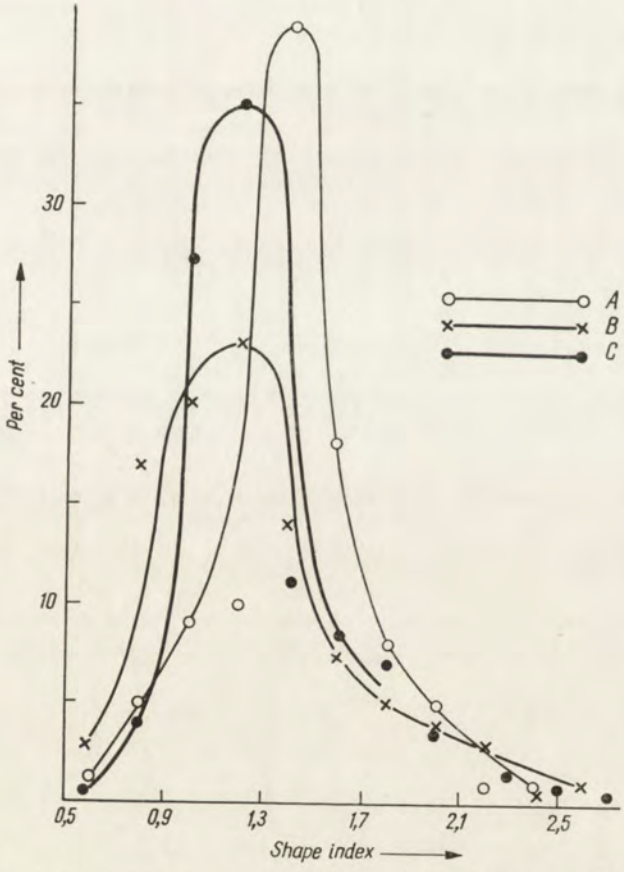


Fig. 3. Distribution of the value of the shape index of *T. vaginalis* in asymptomatic (A), acute (B) and chronic (C) infection

index value round the mean while low plus value of the coefficient of skewness points to a slightly left asymmetry.

The distribution of the value of the shape index of *T. vaginalis* in symptom-free, acute, and chronic infection is illustrated in Fig. 3.

It should be underlined that the data on the *T. vaginalis* shape obtained from biometric measurements have confirmed author's clinical observations that protozoa of various shape are countered in the female vaginal discharge but more than 50 per cent them is of the same definite shape.

Figures 4 to 8 may illustrate the shape of *T. vaginalis* from different clinical forms of infection and from culture. It can be seen from Fig. 4 that the undulating membrane is not always observed and that it may be differently arranged. The parasites whose shape index is greater than 3 have a hidden axostyl. Some of them resemble other flagellata in shape. The observation of some details of the trichomonad structure in direct preparations was possible only with the aid of phase contrast microscope and immersion (×900 to 1500). This also refers to direct preparations of the protozoa from culture. In these preparations trichomonads of various shape were also seen.

There is in Figs. 5, 6, and 7 a series of drawings of the protozoon in the smears from asymptomatic, acute, and chronic infection, arranged according to the value of the shape index; this was made to illustrate the forms used for biometric measurements. Deformed trichomonads with an obvious translocation of the organellae were omitted. Such individuals from different forms of infection to which usual criteria for measurement cannot be applied are presented in Fig. 8.

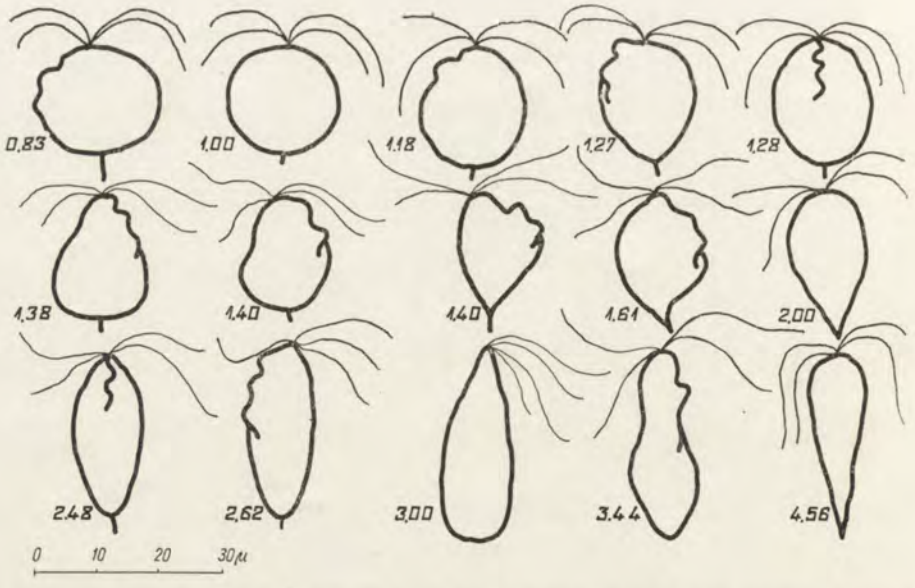


Fig. 4. The shape of *T. vaginalis* in the direct preparations of the vaginal discharge. The numbers on Figs 4—7 mean the shape index

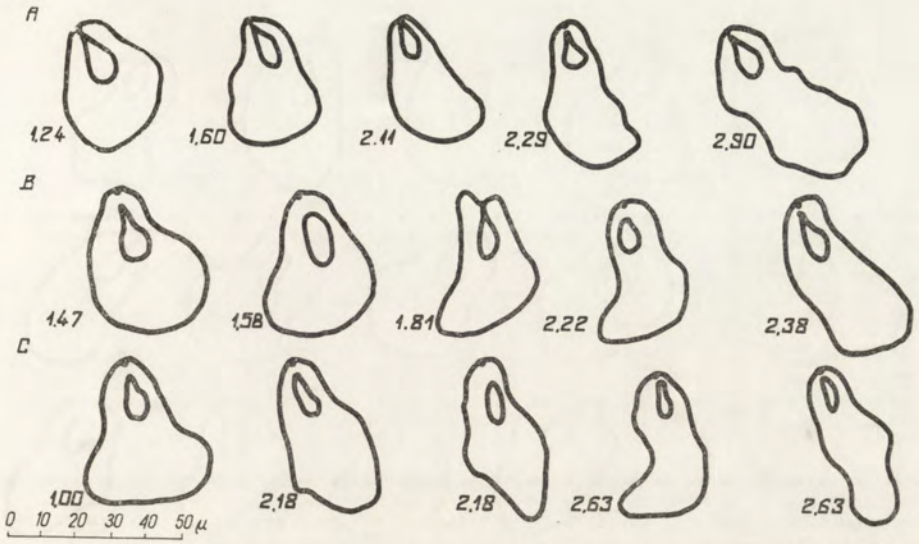


Fig. 5. The shape of *T. vaginalis* in the smears of the vaginal discharge from 3 females with asymptomatic infection

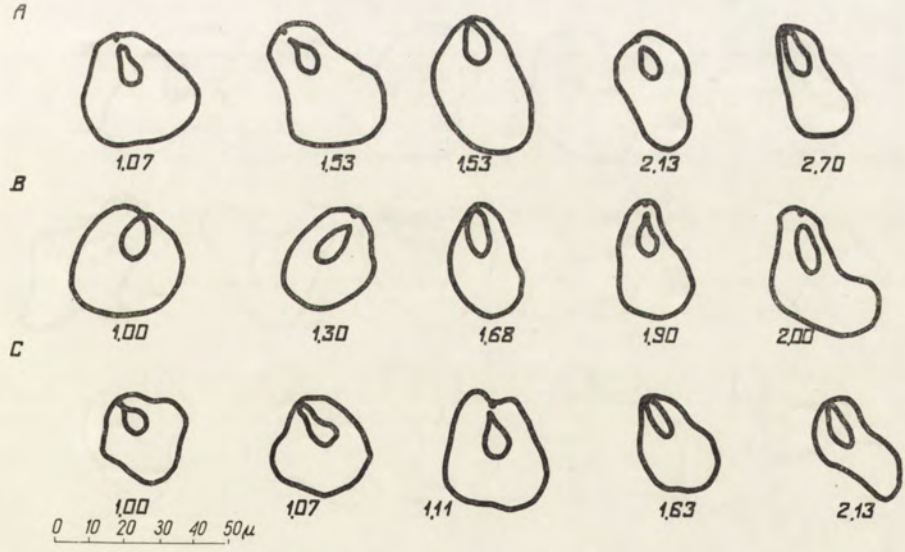


Fig. 6. The shape of *T. vaginalis* in the smears of the vaginal discharge from 3 females with acute infection

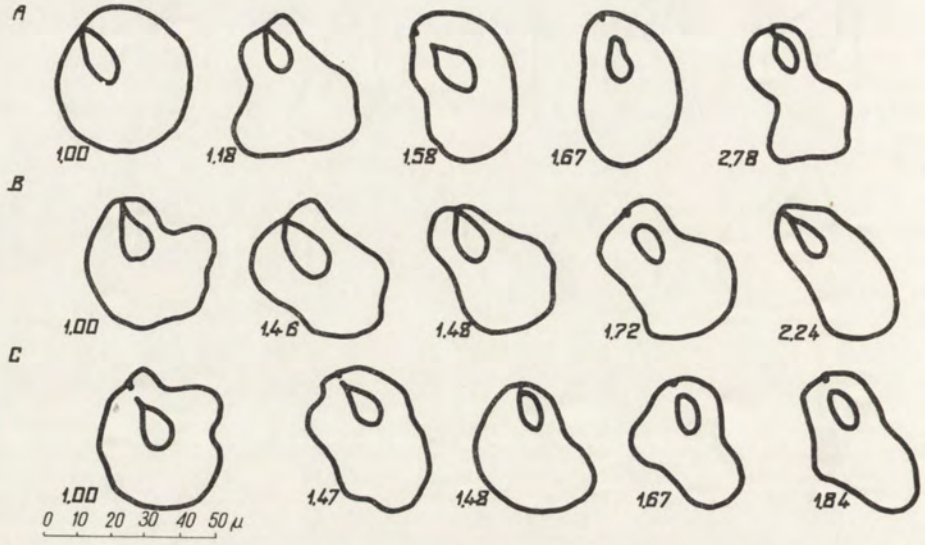


Fig. 7. The shape of *T. vaginalis* in the smears of the vaginal discharge from 3 females with chronic infection

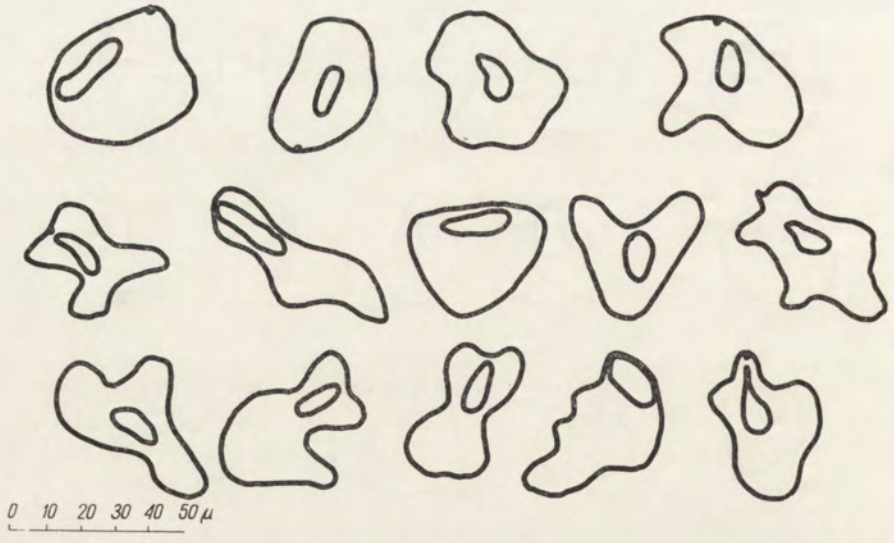


Fig. 8. The abnormal shape of *T. vaginalis* in smears of the vaginal discharge in different forms of infection

It can be seen from the drawings presented in Figs. 4 to 8 that the length and width of *T. vaginalis* not always permit the conclusion on its real size. Often the latter may be more accurately estimated by the measurement of the surface of the cell projection — Table 3 and 4. It is shown in these Tables that the surface of the cell projection in direct preparations was largest in chronic infection and smallest in asymptomatic one. The differences in the mean surface of the protozoon from various forms of infection are statistically significant ($p < 0.01$). It results from Table 4 that in stained preparation the surface was largest in chronic infection and smallest in acute one. The difference between the means of the protozoon surface in different forms of infection is statistically significant ($p < 0.01$).

Table 3

Biometric features of *T. vaginalis*; stained preparations of the vaginal discharge from females with asymptomatic (A), acute (B), and chronic (C) infection

Feature	Cell surface			Cell volume		
Form of infection	A	B	C	A	B	C
Zone of variation	14.5 — 200.0 μ^2	7.5 — 192.5 μ^2	14.5 — 217.5 μ^2	50 — 2800 μ^3	25 — 2500 μ^3	50 — 3000 μ^3
Mean	62.1 μ^2	49.1 μ^2	71.8 μ^2	340.2 μ^3	201.1 μ^3	228.8 μ^3
Standard deviation	±20.0 μ^2	±15.7 μ^2	±17.4 μ^2	±66.9 μ^3	±62.4 μ^3	±82.6 μ^3
Standard error	±0.90	±0.71	±0.78	±8 70	±7.31	±3.94
Mode	59.2 μ^2	51.1 μ^2	68.5 μ^2	325.9 μ^3	213.1 μ^3	225.1 μ^3
Median	61.3 μ^2	51.0 μ^2	70.1 μ^2	337.2 μ^3	207.2 μ^3	227.9 μ^3
Coefficient of skewness	+0.15	−0.13	+0.19	+0.21	−0.19	+0.04
Coefficient of excess	+0.09	−0.11	−0.07	+0.13	−0.17	−0.13
Coefficient of variation	31.6%	32.0%	24.3%	19.7%	31.0%	36.0%

It is shown in Table 3 that considerable differences in the mode, median and mean of the cell surface exist between individual forms of infection which proves an asymmetric distribution of the discussed feature. A high plus value of the coefficient of skewness allows to think of a considerable left asymmetry; the coefficient of excess in asymptomatic and acute infection proves some flattening of the curve of distribution while in chronic infection it testifies to a considerable gathering round the mean value.

On the other hand the distribution of the value of the nucleus surface (Table 4) shows a left asymmetry except for chronic infection.

The cell surface to nucleus surface ratios in various forms on infection

Variation in plasma-nucleus index

The cell surface to nucleus surface ratios in various forms on infection are given in the last columns of Table 4 in the form of plasma-nucleus surface index. It results from this Table that mean value of this index for asymptomatic infection differs from those found in other forms of infection, the

Table 4

Biometric features of *T. vaginalis;* direct preparations of the vaginal discharge from females with asymptomatic (A), acute (B), and chronic (C) infection

Feature	Cell surface			Nucleus surface			Plasma-nucleus index		
Form of infection	A	B	C	A	B	C	A	B	C
Zone of variation	14.5— 217.5 μ^2	14.5— 217.5μ^2	14.5— 217,5μ^2	1.7— 20.9μ^2	0.6— 12.2μ^2	0.6— 11.0 μ^2	6.0— 28.0	6.0— 26.0	2.5— 22.0
Mean	84.3 μ^2	57.2μ^2	93.5μ^2	6.1μ^2	4.3μ^2	6.2 μ^2	14.14	12.71	12.34
Standard deviation	±35.1μ^2	±33.5μ^2	±24.6μ^2	±2.5 μ^2	±2.1 μ^2	±2.0 μ^2	±4.68	±4.45	±4.09
Standard error	±1.39	±1.33	±0.99	±0.12	±0.06	±0.06	±0.57	±0.54	±0.49
Mode	72.5 μ	45.7 μ	80.8 μ	6.1 μ	4.6 μ	6.1 μ	9.63	9.49	9.97
Median	78.4 μ	51.3 μ	88.1 μ	5.9 μ	4.2 μ	5.7 μ	12.90	11.88	12.01
Coefficient of skewness	−0.34	−0.34	−0.52	−0.02	−0.14	−0.06	−0.96	−0.73	−0.58
Coefficient of excess	−0.15	−0.09	−0.15	0.07	−0.60	−0.17	−0.01	−0.50	−0.65
Coefficient of variation	41.6%	58.6%	26.3%	40.6%	48.0%	32.7%	33.1%	35.0%	33.1%

difference being statistically significant ($p < 0.05$). On the other hand the difference in the mean plasma-nucleus index for acute and chronic infection is statistically insignificant. The mode is similar in all forms of trichomoniasis and equals about 10. The projection of the surface of the trichomonad nucleus equals in majority of protozoa to one tenth of the projection of the surface of the whole cell. The differences between the means and modes, and the value of the coefficient of skewness suggest that the distribution of the values of the plasma-nucleus surface index is asymmetrical (left asym-

metry) in all forms of infection. Diagrams of variation presented in Figure 9 may illustrate the distribution of this feature.

The plasma-nucleus surface index of *T. vaginalis* is according to the present author of great importance for the distinguishing the protozoon cells from other elements in smears from the discharges from the vagina, cervix uteri, and urethra. In addition it may be important for the determination of morphological changes in the protozoon cell due to action of chemical compounds. This was seen when the biometric features of *T. vaginalis* from the vaginal discharges of chronic cases treated with arsenic compounds and controls were compared (Table 5). The difference in the means of all the features tested between these two groups is statistically significant ($p < 0.01$)

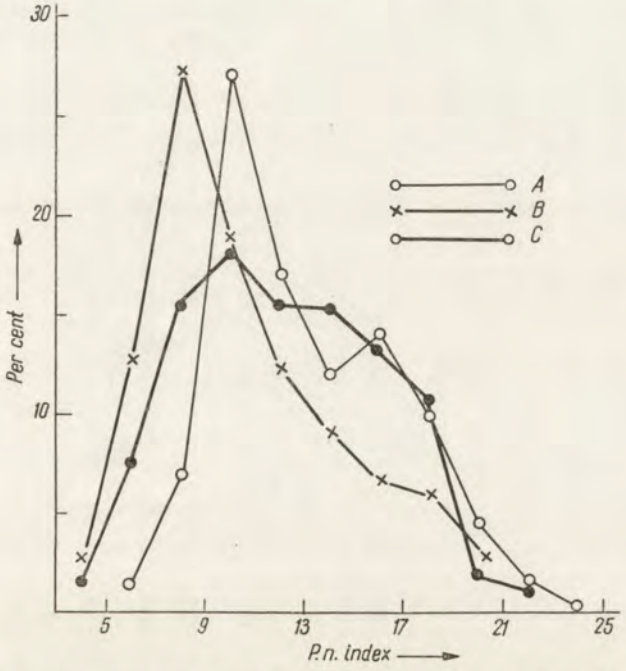


Fig. 9. Variation of the plasma-nucleus index of *T. vaginalis* in asymptomatic (A), acute (B) and chronic infection

except for the average values of the nucleus surface. Thus the nuclei of *T. vaginalis* from females treated with arsenic compounds are of the same size as those from non-treated females while the whole cell becomes distinctly smaller. The value of the plasma-nucleus index points to the fact that the nucleus occupies on the average one fifth of the surface and in some cells may occupy even one third of it.

The volume of *T. vaginalis* in direct preparations was calculated from the length and width according to the above given formula of Oliva et al. (Table 3); it ranged from 25 to 3000 μ^3. The distribution of this feature is

slightly asymmetrical with a right asymmetry in acute infection and left asymmetry on other forms of infection.

The second formula (K a w i a k) was applied for the calculation of *T. vaginalis* volume in stained preparations because the length and width of the protozoon in such preparations express the real size to a much lesser degree than in direct preparations — Table 6.

The volume of *T. vaginalis* cell ranged from 50 to 3000 μ^3 in stained preparations from the vaginal discharge.

Table 5

Biometric features of *T. vaginalis*; stained preparations of the vaginal discharge from females with asymptomatic (A), acute (B), and chronic (C) infection

Feature	Length	Windth	Shape index	Celi surface	Nucieus surface	Plasma-nucleus index
Zone of variation	4.3— 14.5 μ	4.3— 9.7 μ	0.6— 2.6	7.5— 159.5 μ^2	1.7— 12.2 μ^2	2.5— 17.5
Mean	10.2 μ	9.4 μ	1.09	42.7 μ^2	6.2 μ^2	5.18
Standard deviation	$\pm$3.5μ	$\pm$2.1 μ	$\pm$0.26	$\pm$12.1 μ^2	$\pm$1.1 μ^2	$\pm$1.06
Standard error	$\pm$0.21	$\pm$0.13	$\pm$0.02	$\pm$0.73	$\pm$0.07	$\pm$0.06
Mode	15.7 μ	14.4 μ	1.11	33.0 μ^2	6.1 μ^2	5.93
Median	16.1 μ	15.1 μ	1.10	41.7 μ^2	6.2 μ^2	5.15
Coefficient of skewness	+0.17	+0.59	−0.08	+0.38	+0.11	−0.70
Coefficient of excess	−0.13	+0.07	+0.09	−0.16	−0.13	+0.10
Coefficient of variation	21.3%	14.1%	23.9%	28.7%	18.1%	20.5%

The mean volume of the parasite was largest in chronic infection and smallest in acute disease. The difference in the mean volume for various groups is statistically significant ($p < 0.01$). The differences in the value of the mean, mode, and median, and the coefficient of skewness allow to state a left asymmetry of small degree with a considerable dispersion round the average values.

The *T. vaginalis* plasma-nucleus volume index in chronic infection shows the distribution very similar to the normal one. In acute and asymptomatic infection the data demonstrate significant left asymmetry.

Table 6

Biometric features of *T. vaginalis*; stained preparations of the vaginal discharge from females with asymptomatic (A), acute (B), and chronic (C) infection

Feature	Cell volume			Nucleus volume			Plasma-nucleus index		
Form of infection	A	B	C	A	B	C	A	B	C
Zone of variation	100—2500 μ^3	50—2500 μ^3	50—3000 μ^3	5—65 μ^3	5—45 μ^3	5—100 μ^3	7.5—37.5	7.5—40.0	7.5—42.5
Mean	343 μ^3	309 μ^3	386 μ^3	13.6 μ^3	12.2 μ^3	14.3 μ^3	24.16	26.49	25.16
Standard deviation	$\pm$52.3μ^3	$\pm$43.2μ^3	$\pm$54.1μ^3	$\pm$4.3 μ^3	$\pm$3.7μ^3	$\pm$4.6 μ^3	$\pm$6.16	$\pm$5.83	$\pm$8.11
Standard error	$\pm$6.12	$\pm$5.72	$\pm$0.16	$\pm$1.73	$\pm$1.49	$\pm$1.82	$\pm$2.46	$\pm$2.33	$\pm$3.24
Mode	338.2 μ^3	301.9 μ^3	372.1 μ^3	12.4 μ^3	11.1 μ^3	13.2 μ^3	20,41	23.16	24.97
Median	330.3 μ^3	311.5 μ^3	379.7 μ^3	12.0 μ^3	11.6 μ^3	13.1 μ^3	23.13	22.99	25.01
Coefficient of skewness	−0.09	−0.17	−0.27	−0.28	−0.28	−0.23	−0.60	−0.57	−0.02
Coefficient of excess	−0.19	−0.23	−0.27	−0.03	−0.07	−0,11	−0.13	−0.22	−0.07
Coefficient of variation	15.5%	13.9±	14.0%	31.7%	30.7%	32.0%	25.4%	22.0%	32.2%

Summary

The biometric features of *Trichomonas vaginalis* from the vaginal discharge of females with various stages of trichomoniasis were studied in direct smears and stained preparations. The mean length, width, and surface of the parasite are larger in stained than in direct preparations. Irrespective of the kind of preparations there are statistically significant differences in the length, width, surface of the projection of the cell and that of the nucleus, volume, and shape index of *T. vaginalis* from asymptomatic, acute, and chronic infection. On the other hand the value of the plasma-nucleus index is similar in these groups except for the protozoa from females with chronic infection treated with arsenic compounds where the value for this index is several times smaller.

In the majority of groups tested the asymmetric distribution of the biometric features was obtained (left asymmetry); this means that in majority of protozoa the value of the features tested was lower than the mean.

After one-month cultivation no statistically significant differences in the mean length, width, and shape index were found in populations of *T. vaginalis* from females with asymptomatic, acute, and chronic infection.

STRESZCZENIE

Ustalono cechy biomeryczne *Trichomonas vaginalis* pochodzących od kobiet w różnych stanach rzęsistkowicy. Badań dokonywano na bezpośrednich rozmazach i na preparatach trwałych. Ustalono, że średnia długość, szerokość oraz powierzchnia rzęsistka pochwowego w preparatach trwałych jest większa niż w preparatach bezpośrednich. Nie zależnie od rodzaju preparatów istnieją znamienne statystycznie różnice między długością, szerokością, powierzchnią rzutu komórki i jądra, objętością oraz wskaźnikiem kształtu *T. vaginalis* w wydzielinie pochwowej kobiet w różnych stanach rzęsistkowicy, a mianowicie w zarażeniu bezobjawowym, ostrym i przewlekłym. Natomiast wartość wskaźnika plazmojądrowego jest w tych grupach zbliżona. Tylko u pierwotniaków uzyskanych od kobiet z zarażeniem przewlekłym, leczonych związkami arsenowymi, wartość tego wskaźnika jest kilkakrotnie mniejsza.

W większości grup uzyskano asymetryczny rozkład cech biometrycznych (asymetria lewa), co oznacza, że wartość badanych cech u większości pierwotniaków była niższa od uzyskanych wartości średnich. Po jednomiesięcznej hodowli nie stwierdzono statystycznie znamiennych różnic w średnich wartościach długości, szerokości i wskaźnika kształtu między szczepami uzyskanymi od kobiet zarażonych bezobjawowo oraz z rzęsistkowicą ostrą lub przewlekłą.

REFERENCES

Doflein F. und Reichenow E. 1949—1953: Lehrbuch der Protozoenkunde, G. Fischer, Jena.

Hill A. B. 1962: Statystyka dla lekarzy, PWN, Warszawa.

Jirovec O. 1962: Parasitologie für Ärzte, G. Fischer, Jena.

Kawiak J. 1959: Zmiany wielkości jąder fibroblastow w hodowli tkanek, Folia morphol. 10, 29—37.

Kudo R. R. 1954: Protozoology, Thomas, Springfield, Illinois.

Kurnatowska A. 1965: Studies on *Trichomonas vaginalis* Donné. Part I. Clinical aspects and pathogenesis of Trichomoniasis of the female urogenital organs. Stainig of *Trichomonas vaginalis* in the diagnosis of Trichomoniasis, Acta med. pol. 6, 4, 439—469.

Kurnatowska A. 1966: Studies on *Trichomonas vaginalis* Donné. Part II. The sensitivity of *Trichomonas vaginalis* on the new halogenosulphonamides in vitro and the effect of the treatment of trichomoniasis by new drugs. Folia med. Lodzensis (in press).

Okła I. 1954: Rzęsistkowe zapalenie pochwy i jego leczenie, PZWL, Warszawa.

Szulc S. 1961: Metody statystyczne, PWE, Warszawa.

EXPLANATION OF PLATES I — II

Trichomonas vaginalis Donné

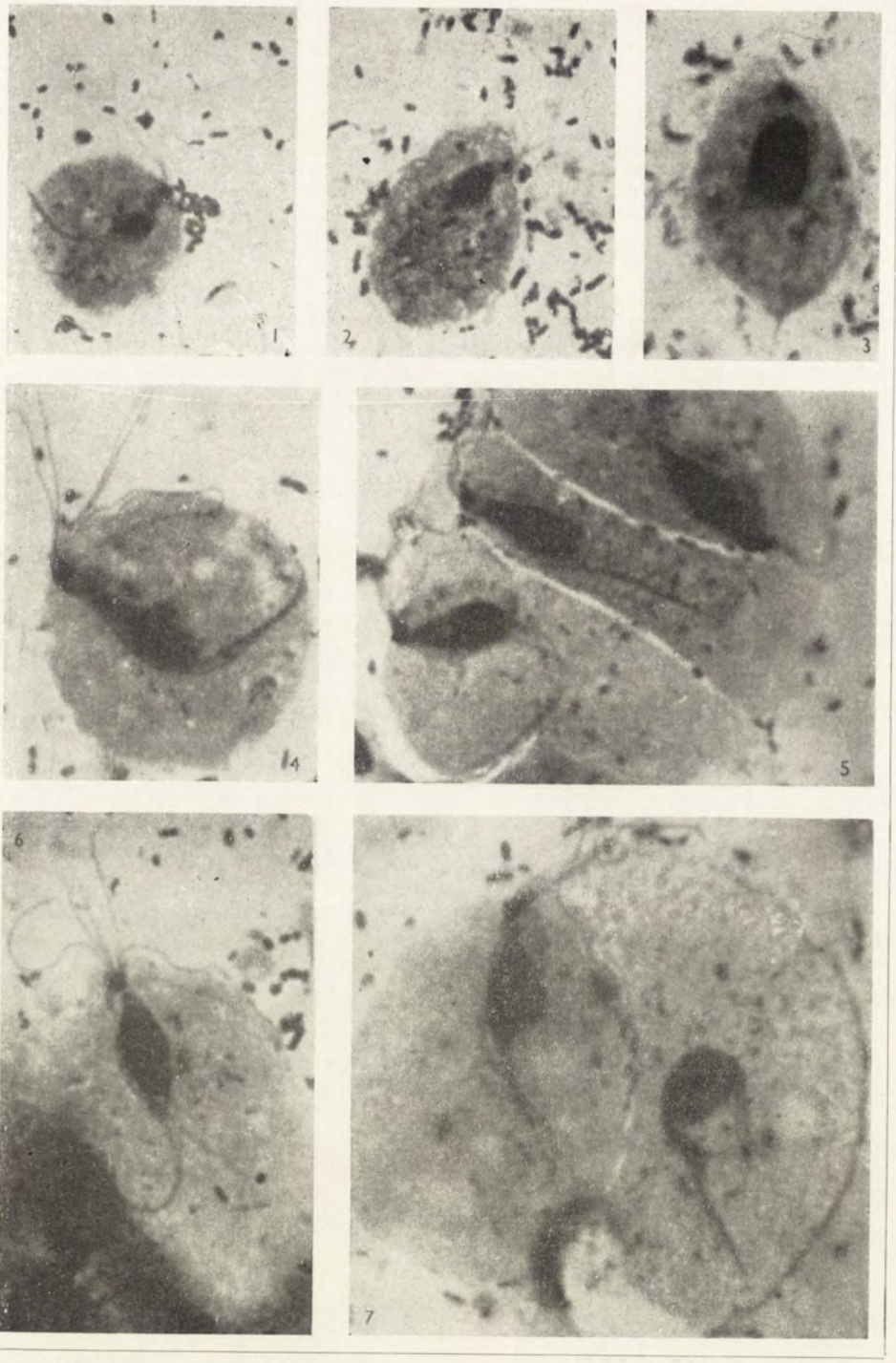
1—3: Trichomonads of round, pear-like and ovoid shape from a one-month culture. Bouin — Allen; Wright, 1500×, immers. 4: Trichomonad of round shape in symptomless infection. Sublimate, pyronine-methyl green. 2000×, immers.

5—9: Vaginal trichomonads in symptomless infection with visible deformations caused by mutual adhesion of cells to one another and to epithelium or by the polar flattening. Bouin — Allen; Wright, 2000×, immers.

10: Vaginal trichomonads in acute infection partly deformed by mutual adhesion. Sublimate; Mayer's carmalum. 2000×, immers.

11—12: Vaginal trichomonads of fusiform and ovoid shape in chronic infection. Methanol; Wright, 1000 and 2000×, immers.

13—14: Vaginal trichomonads in chronic infection prior and after application of arsenic compounds. Changes in the size ratio nucleus: cell of the protozoon, are seen. Methanol. Wright. 2000×, immers.



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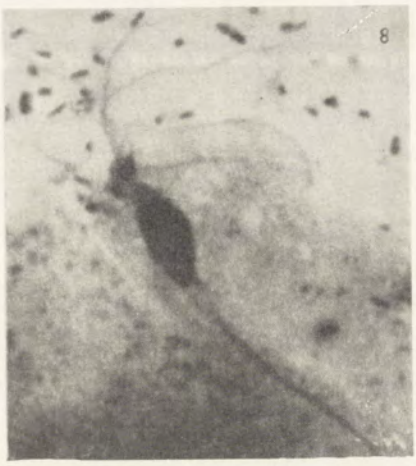

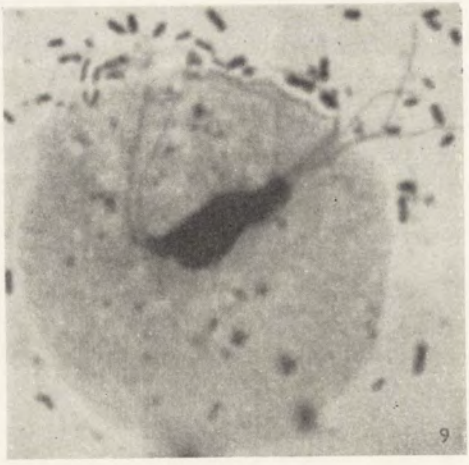

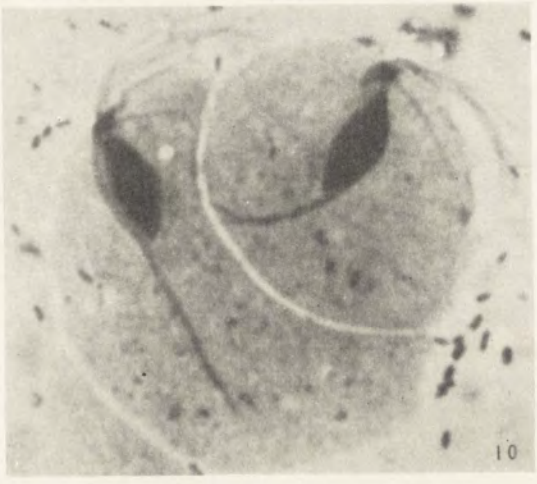

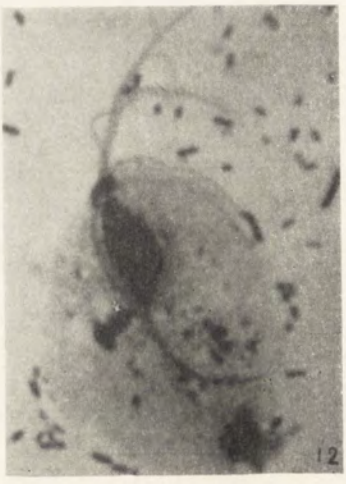

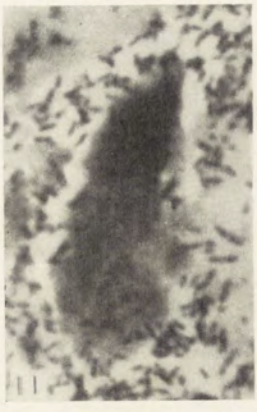

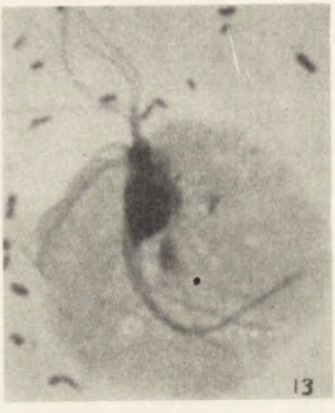

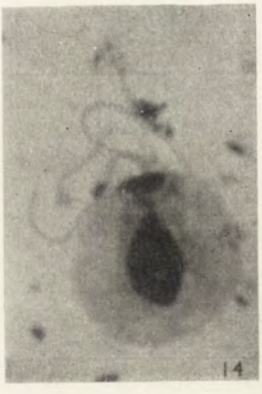

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Membrane folding and plasma-membrane ratio in the movement and shape transformation in *Amoeba proteus*

Fałdowanie błony i stosunek plazmo-membranowy w zjawiskach ruchu i przemianach kształtu u *Amoeba proteus*

The behaviour of the cell membrane, or rather the cell surface structures, is of great importance for understanding the amoeboid movement. This had been notoriously stressed even by the early authors which were not aware of the fine structure of the so called plasmalemma in amoebae.

The cell wall of such an amoeba as *Amoeba proteus* consists of a basal layer about 80 Å thick (Pappas 1954) which might be comparable to the unit membrane of other living cells, and of an outer mucus sheet measuring up to 2000 Å, endowed with numerous hair-like extensions (Schneider und Wohlfarth-Bottermann 1959, Brandt and Pappas 1960, O'Neill 1964). Both these structures will be denoted, in this article, together as cell membrane or cell surface material (reserving the term of the unit membrane for the membrane itself), because — as this has been already pointed out by Wolpert, Thompson and O'Neill 1964 — it would be rather unlikely to expect their independent motory behaviour during the cell locomotion.

Material used for this study was *Amoeba proteus* cultured in the Chalkley medium following the procedure applied in the Zoology Department, Kings College, London, and fed with *Tetrahymena* and *Colpidium* grown in yolk cultures.

Special thanks are due to Dr. C. Chapman-Andresen (Carlsberg Laboratory, Copenhagen) and to Dr. A. L. Judin (Institute of Cytology, Leningrad) for providing us with initial strains of amoebae.

Outline of the theories

Current views on the behaviour of cell surface

All the theories of the behaviour of cell surface in amoebae may be divided in two main cathegories depending on their attitude towards the problem of permanency of the cell membrane. Some authors believe the cell membrane to undergo a rapid resorption-restoration cycle related to the movement and transformation of cell shape, while anothers consider the membrane as a struc-

ture relatively permanent which renewal is so slow that it should be neglected as a factor of locomotion.

Two opposite views had been put forward by the adherers of the first concept. Schaeffer 1920 suggested that the cell membrane is being constantly resorbed in the anterior part of amoeba and restored at the same rate in the posterior end. If it was true, the cell surface should move forwards over the cell body, i.e. it should move in respect to the ground at a speed exceeding the cell locomotion. The view of Schaeffer 1920 had no followers and it will be postponed in further considerations.

A quite contrary view on the resorption and restoration of the cell membrane has been expressed by Goldacre and Lorch 1950 and developed in full details by Goldacre 1961. In Goldacre's opinion, the cell membrane in a moving amoeba is resorbed in the tail and formed de novo at the front. The rates of resorption and restoration are approximately equal and precisely co-related to the speed of locomotion, in such a manner that exactly the same amount of the new membrane material arises at the tip of extending pseudopod which is

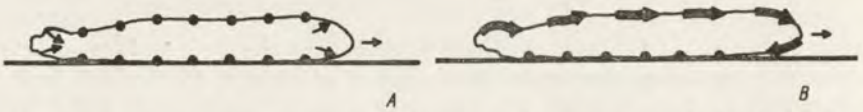

Fig. 1. Diagrammatic representation of two main concepts of the behaviour of cell surface during amoeboid movement: the theory of stationary condition of cell membrane (A) and the theory of membrane rolling (B). The arrows and solid circles on the membrane indicate its motory behaviour, arrow at the front of the cell — the speed of its locomotion, fine arrows inside the cell in A — the postulated sites of membrane formation and resorption

necessary to cover its growing area, and — respectively — the same amount which becomes excessive because of the tail concentration is resorbed there. As a result, there is no necessity for the membrane to move at all, and in fact, it remains at rest along the sides of the body. In other words, the membrane around the whole cell would be stationary in relation to the ground, whereas in relation to the cell it would move backwards at a speed equal to the rate of cell locomotion (Fig. 1 A).

The theory of Goldacre seems to be supported by many authors, Bell 1961, Champan-Andresen 1964, Jahn 1964, and—in the case of cellular slime molds — by Shaffer 1963. It has also been preferred among others by one of the present authors, in a review article (Grębecki 1964). As to the mechanism of the membrane resorption and restoration, it might be conceived to be molecular as postulated by Goldacre 1952, or based on the turnover of formed membraneous structures between the cell surface and the endoplasmic reticulum as suggested by Wohlfarth-Bottermann 1964.

The obvious postulation of the second group of theories, considering the membrane to be a rather permanent structure, is that the membrane should move forwards together with the whole cell, and the total average rate of the membrane movement should be equal to the rate of locomotion. In other case the cell could not move at all. However, the distribution of the membrane shifts may be different in various regions of a moving amoeba, and this involves a possibility of different interpretations.

The classical concept of the behaviour of cell membrane in amoebae, as put forwards by Jennings 1904 and supported by Mast 1926, currently called the theory of membrane rolling, belongs to this group. It is presumed that the membrane rolls over the cell body as a caterpillar over the wheels. This means that the cell surface behaves in quite an asymmetrical manner, on the upper and lower side of the body. The lower surface, adhering to the substrate, would be stationary in relation to the ground, whereas the upper surface — on the contrary — should compensate the difference by shifting forwards in relation to the ground at a double speed of cell movement (Fig. 1 B). This means that, in relation to the cell, the lower surface would shift backwards and the upper — forwards, both at the same speed equal to the rate of locomotion. In other words, the cell membrane should constantly roll over the cell body in the vertical plane.

The theory of membrane rolling was predominant in the past decades, and even in the recent years it met further support in some studies, e.g. in these of Griffin and Allen 1959 and Abé 1962. Also Seravin 1964, in his review of the subject, seems to agree with the rolling of the cell membrane rather than with its stationary condition.

Some other possibility of shifting the permanent cell membrane together with the moving cell had been put forward in the recent years, as well for amoebae themselves, as for other cells which manifest the amoeboid movement. Holtfreter 1947 suggested that the membrane of some cells of the amphibian embryos moves with the cell owing to an elastic extension in some areas which is followed by a subsequent return to the former state, and to a next stretch in another site. Ambrose 1961 proved another mechanism for the movement of fibroblasts, based on the undulatory movement of the lower surface of the cell which adheres to the substrate. Finally, Wolpert (Wolpert, Thompson and O'Neill 1964, Wolpert 1965) put forward the view that the material of surface structures in amoeba is fluid and flows forward, following in such a way the locomotion of the whole cell.

The theories of Holtfreter, Ambrose and Wolpert agree with the classical view in this respect that they all postulate a rather permanent character of the cell membrane, and — as a consequence — they should presume that the total average rate of membrane shift is equal to the rate of cell locomotion. On the other hand, they do not postulate any essential asymmetry of the membrane movement — as the rolling theory does.

Membrane folding, a new hypothesis

The hypothesis of the behaviour and properties of cell surface in amoeba which is put forward by the present authors in this study, may be characterized in the following points:

1. The cell membrane in amoeba is a rather permanent structure, and its renewal is too slow to play any role in the locomotion.

2. The membrane shifts forwards together with the whole cell, with a total average velocity equal to the rate of locomotion.

3. Asymmetry of membrane shift may arise in different areas of the cell surface, as a secondary phenomenon, not as a rule.

4. The membrane shifts forwards owing to the simultaneous or alternating processes of expansion in some areas and collapse in anothers.

5. The cell membrane is non-extensible without affecting its structure, i.e. the expansion of surface cannot be due to a mechanical stretch.

6. The cell mebrane in amoeba is distinctly convoluted, and therefore the expansion of surface may be explained by its unfolding and the collapse — by re-folding.

The first postulation became undisputable since the experiment of Wolpert and O'Neill 1962 proving that the half-life period of the surface layer in *Amoeba proteus* lasts about 5 hrs., whereas this organism needs only a few minuts to pass its own length. The second postulation, as it has been pointed out above, is nothing else but a logical consequence of the former one.

The third and the fourth postulation rank our point of view to the same cathegory with the theories of Holtfreter 1947, Ambrose 1961, and Wolpert 1965, and — on the other hand — they stress the difference between our concept and the classical theory of membrane rolling.

The fifth postulation needs a short discussion. By considering the cell membrane to be non-extensible we mean that it is not capable to extend its area when mechanically stretched by the moving cell. In this, our view contradicts that of Holtfreter 1947. It seems rather obvious that no extension could be imagined without any re-arrangement of the compactly packed lipid molecules of the unit membrane. This view meets also some experimental support. Ponder 1948 found that no further swelling is possible in the erythrocytes which had reached a spherical form. Recently, the elasticity of the body wall in *Amoeba proteus* has been directly measured by suction method, by Kanno 1964. This author concludes that the elasticity is rather due to the ectoplasm layer beneath the membrane and he states: "plasmalemma did not take any important part in elasticity of surface structure". It should be noticed here that the view on the elasticity of cell membrane expressed by Wolpert, Thompson and O'Neill 1964 is very similar to our own.

If the cell membrane is non-extensible, and if it cannot be rapidly supplemented from inside the cell, the deficient amount of the surface material, necessary to cover the expanding regions of the body, should be transported from other areas of the cell surface. In this essential point our concept coincides with the theory of membrane flow put forward by Wolpert. However, there are two reasons restraining us to accept the Wolpert's theory in its whole extent: 1. Fluidity of the surface structures in amoeba does not seem to be well established. The fluid nature of the outer mucus sheet is indeed rather obvious. Semifluidity of the inner lipid leaflets of the unit membrane could be eventually explained as any kind of the molecular shearing, but the fluid properties of the protein network seem hardly imaginable. 2. There is no evidence what is the kind of driving force which could propel such a liquid stratum forwards, over the cell body.

For that reasons the present authors tend to substitute the idea of membrane flow by a more concrete mechanism of membrane folding and unfolding, as put forward in the sixth postulation of the present hypothesis. The fact that the cell surface of amoeba is higly convoluted, and its folding is much more distinct in the posterior region of the body than on the surface of new pseudopods, is commonly known and easy observable even in the light microscope (Pl. XIV 63). That means that a stock of an "excessive" material is always present on the cell surface which can unfold, shift forwards, and cover the expanding area of growing pseudopods. This reserve stock of the surface ma-

terial is constantly regenerated by re-folding of the membrane which steadily occurs in the tail and in the contracting pseudopods.

The basic mechanism of the membrane movement by folding and unfolding is diagrammatically represented by the Fig. 2. The cell is presumed to be cylindrical. At the first picture the system is represented at the stage of its maximal folding, manifested mainly by the numerous folds in the posterior part. The streaming of cytoplasm directed forwards stretches the membrane at the front which results in its unfolding and shifting forwards. The stage of the maximal unfolding is seen in the next drawing. The process of the posterior contraction re-folds the membrane at the rear end, and the previous state of membrane is restituted, but the cell — as well as its membrane — are clearly shifted forwards together (the last picture in the Fig. 2).

Of course, the scheme shown in the Fig. 2 should be considered as representing a rather idealized system, not the true amoeba, even not a monopodial one. It could be presumed, for example, that the stages of unfolding and re-fol-

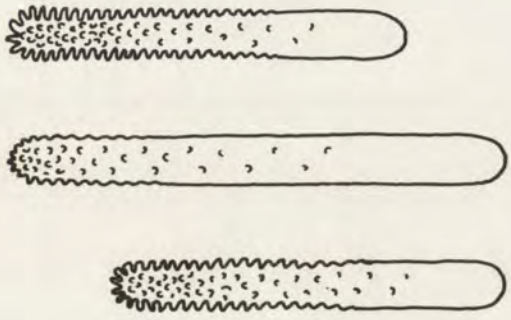

Fig. 2. Scheme of the presumed basic mechanism of membrane folding and unfolding as represented on a model cylindrical body. Unfolding occurs between the first and the second stage, and the re-folding — between the second and the third ones

ding alternate very quickly and overlapse in time, which should result in the undulations of the cell surface similar to those which were stated by A m b r o s e 1961 in fibroblasts. In such a case the movement of the whole monopodial cell with its membrane might be roughly compared to the movement of an earth-worm.

It seems that in fact, the processes of folding and unfolding are rather simultaneous and do not represent any separate stages, because as well the extension of a pseudopod and unfolding of its membrane, as the refolding of membrane on the tail, depend both on the same process of the intracellular contraction. Nevertheless, an alternation of some maxima of unfolding (=cell elongation) and re-folding (=cell shortening) could be expected. Such a rhytmicity of movement had been indeed found by K a n n o 1965, and it has been registered photographically in the present study (Pl. XIV 64).

The theory of membrane folding and unfolding seems to explain in very simple manner the behaviour of polypodial forms of amoebae, which are in

Amoeba proteus the most common ones. The mechanism of unfolding and re-folding, in this case, is shown in the Fig. 3. Extension of a pseudopod enlarges its area, streches the membrane, and unfolds it. Then the unfolding surface material shifts forwards from another regions of the body. When no more material capable to unfold is present near the pseudopod, the membrane stretching reaches its maximum and any further extension of the pseudopod becomes impossible. Then it stops and a new pseudopod protrudes from another area which is rich in the folded surface material. The stock of the folded membrane is still restituted in the tail and in the old pseudopods which are contracting.

It seems very important that the membrane folds are distributed at random in various directions, because this enables the unfolding to occur also in many directions simultaneously with very little mutual interference. For the same

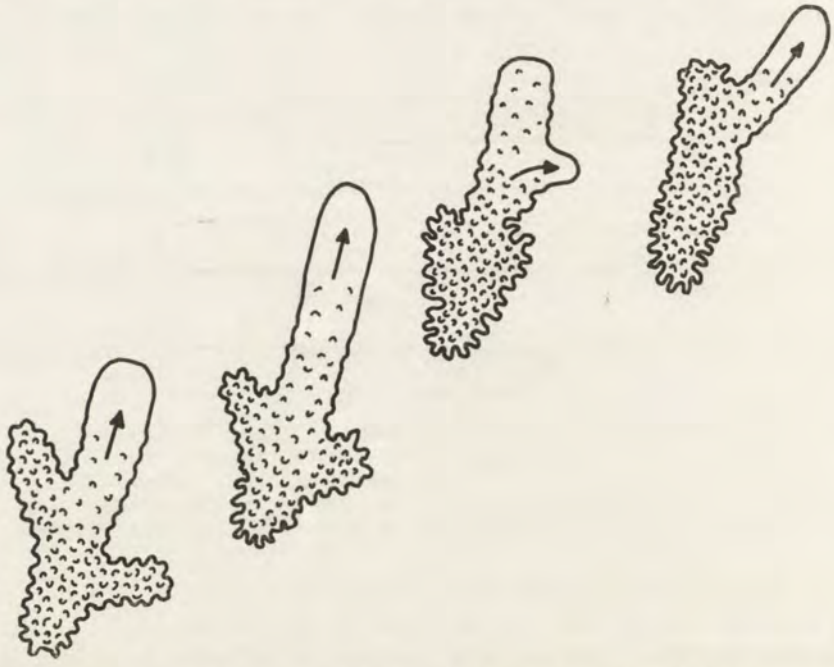

Fig. 3. Diagrammatic representation of the mechanism of substitution of pseudopods in a polypodial amoeba, as suggested by the theory of membrane folding and unfolding. Full explanation in the text

reason the membrane could remain at rest in a definite area but undergo unfolding and shifting beside it. This last point is very essential for the hypothesis because it allows to make the necessary assumption that the cell membrane fails to unfold but remains stationary in that site of the cell which adheres to the substrate.

In the experimental approach, the hypothesis of membrane folding and unfolding was confronted with two greatest current theories: that of membrane rolling and that of stationary condition of the membrane.

Re-examination of some earlier data

Membrane staining experiments

Some vital stains were used in the past years as surface markers to elucidate the behaviour of cell membrane during the locomotion of amoeba. Such an experiment was performed at first by Goldacre and Lorch 1950 with dilute neutral red solution. The authors concluded that amoebae accumulate the dye in their tails while the advancing pseudopods appear colourless, which would speak in favour of the membrane resorption in the tail and its restitution in the front of an actively streaming cell. Prescott 1953 repeated the neutral red experiment and came to similar conclusions, however he has reported also that the dye becomes soon distributed throughout the cytoplasm, being ingested in the small vacuoles. Chapman-Andresen 1964 labelled the cell surface with alcian blue; this stain is bound by the mucopolysaccharide sheet covering the true cell membrane in amoebae. Her conclusions agree also with the view that the cell surface remains stationary during amoeboid movement, and—on the other hand—she describes a phenomenon of ingestion of the stained membrane.

The present experiments were performed with alcian blue solutions. The alcian green and yellow have also been tested but no essential difference in the behaviour of amoebae was stated, and the study was subsequently limited to the alcian blue which allows to obtain the most contrasting stain. A defined volume of the Chalkley medium containing numerous amoebae was mixed with an equal volume of the dye solution (of a concentration twice higher than the required final concentration). After 1—2 min. of incubation some cells were transferred by means of a narrow capillary, with a quantity of liquid as small as possible, into a normal stain-free Chalkley medium, and immediately observed under low power microscope.

After the treatment with low concentration of alcian blue (below 1:10000), as a matter of fact the cell surface seems to be stained on the tail only, while the advancing pseudopods appear colourless. Nevertheless, this result cannot confirm the concept of stationary membrane, for the following reasons:

1. The surface of the rear end of amoeba becomes stained immediately after the cell has been immersed in the experimental solution. It is not necessary to wait until the animals pass their own length, as postulated by Goldacre. The phenomenon is easy to be followed when staining is performed under a continuous microscopic control.

2. The surface of a new pseudopod is in fact apparently stainless, however the blue shade of its membrane turns to be visible just since the pseudopod has been contracted. This phenomenon may be observed in two cases: when the pseudopod becomes a part of the hinder half of the advancing amoeba and therefore it is gradually incorporated into the posterior zone of contraction, and when an anterior pseudopod is stimulated mechanically or by a light beam.

Both observations mentioned above seem to be fairly well explained by the concept. of membrane folding and unfolding. All the cell surface is in fact evenly stained, but—immediately—the shade appears much more intense in the region of highly folded and convoluted membrane, i.e. on the tail, owing to a greater quantity of stainable material there. Extension of a new pseudopod is connected with unfolding of the membrane which results

in an apparent disappearance of the stain. The same area may reveal its colour again if the membrane refolds after a spontaneous or stimulated contraction of the pseudopod.

When higher concentrations of alcian blue (1:10000 and more) are used the impression of dye-accumulation on the tail surface is rather illusory. Very soon after immersion in the experimental solution, pinocytosis sets on and amoebae assume the shape of rosette, as described by Chapman-Andresen 1962. After the transfer into a stain-free medium pinocytosis is interrupted and locomotion reappears but fragments of stained cell membrane, ingested pinocytotically, form a cluster which remains for a long time in the posterior part of cytoplasm (Fig. 4 A). Therefore, after incubation in

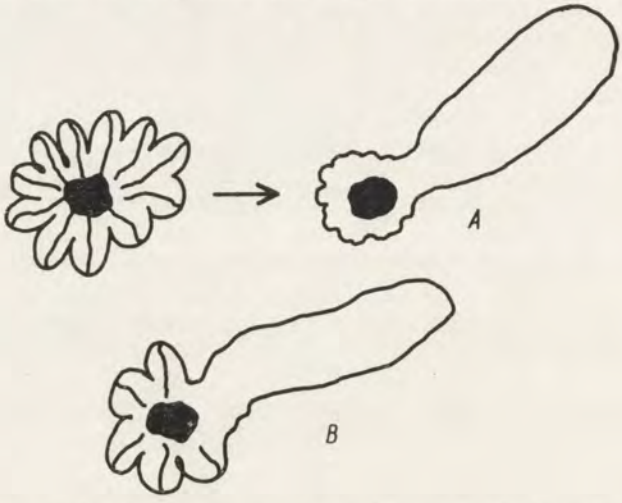

Fig. 4. Origin of the apparent colouration of the tail by alcian blue in the case of pinocytosis. A. Pinocytotic rosette transforming in a monopodial amoeba bearing a clump of ingested membrane inside its tail part. B. Imperfect rosette giving the illusion of absorption of the stain by the tail

higher cocentrations of the dye which induce pinocytosis, the stain is not located on the surface of the amoeba's tail but in its interior, and the experiments has no significance for the theory of stationary condition of cell membrane.

An intermediate situation may also be observed in a few cases, when during pinocytosis amoeba fails to form a full rosette, but one of its pseudopods preserves the locomotory character (Fig. 4 B). Even in such a situation the accumulation of the dye on the tail surface is apparent, because in this case the rear region of animal is constituted by the rosette, i.e. by a system of pinocytotic pseudopods, and not by the tail.

This explanation of the experiments based on staining the cell surface with vital dyes, which is offerred by the membrane folding concept, seems to be in full agreement with the results reported by Wolpert and O'Neill 1962 and Wolpert. Thompson and O'Neill 1964. Wolpert and his

co-workers labelled the cell surface of amoeba with fluorescent antibody, and came to the conclusion that the period of half-life of the surface layer amounts about 5 hrs., i.e. the membrane cannot be formed in the front, resorbed in the tail, and remain stationary on the sides. According the present explanation, the vital stains remain also evenly distributed over the unevenly folded membrane for a long time. In the experiments of W o l p e r t ingestion of the membrane by pinocytosis may interfere gradually but in the same meaning as in ours.

Criticism of the W o l p e r t's conclusions by J e o n and B e l l 1964 may also be disputed in the light of the membrane folding theory, and taking into account the semi-fluidity of the mucopolysaccharide sheet covering the outer surface of cell membrane. J e o n and B e l l 1964 report that if an amoeba labelled with fluoresent antibody is squeezed into a narrow capillary tube, the new pseudopods are not fluorescent and can take new label. As it was proved by O'N e i l l 1964, the antibody in question binds to the mucopolysaccharides. It seems reasonable to postulate that it covers only a thin outer layer of the mucous sheet which is known to be up to 2000 Å thick. As it is schematically presented in the Fig. 5, in a narrow capillary just this labelled layer of

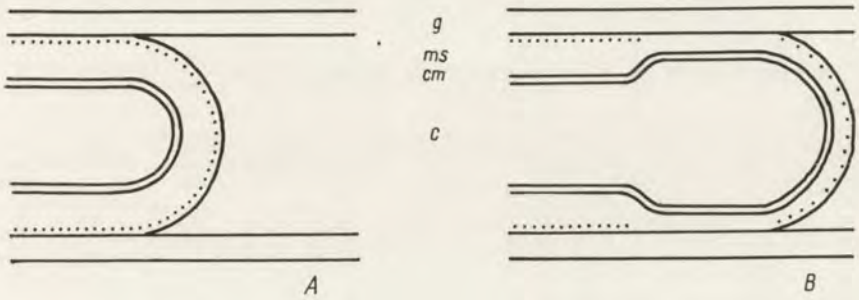

Fig. 5. Tentative explanation of the arising of antibody-free pseudopods when the labelled amoeba is squeezed into a narrow capillary tube; *g* — walls of the glass capillary, *ms* — mucus sheet, *cm* — the proper cell membrane, *c* — the cytoplasm; the outer layer of the mucus sheet labelled by the antibody is dotted. A. Initial stage. B. Further extension of the pseudopod

mucous is pressed against the walls of the tube. When under such conditions, a new pseudopod is formed and its membrane undergoes unfolding, the outer layer of semifluid mucus (which is labelled) should remain adhering to the glass and left behind. Therefore, the new pseudopod protruding in the capillary is probably covered by a mucopolysaccharide sheet which is slightly thinner but is free of the former label and apt to bind new antibodies.

Effect of mechanical obstacles

G o l d a c r e 1961 describes some experiments which may be generally defined as attempts to analyse the behaviour of the cell membrane by impeding or modifying the movement by means of mechanical obstacles. The first experiment consisted in placing few parallel filaments of the glass wool over an amoeba at the right angle in respect to the direction of its movement. The locomotion of the animal still persisted but the position of glass fibres remained unchanged. This result is considered by the author as an evidence proving that the cell surface is stationary.

The experiment quoted above has been repeated by the present authors with the same result. However, the diameter of the glass fibres used in this experiment, as well by Goldacre 1961 as in the present study, was relatively large (25 μ) in respect to the diameter of the amoeba's pseudopods. Shifting of such a load by the subtle movements of the cell surface seems to be in fact hardly expectable. It should be rather postulated that the membrane may freely fold and unfold over all the cell surface except for a longitudinal stripe between the points supporting the fibers (see Fig. 6). In this respect the experiment with glass filaments seems to be comparable to the old experiment of McClendon 1909 who demonstrated the impossibility to stop an amoeba by pinning it down with a needle.

Another experiment was performed in this study in order to test the eventual shifting of such an obstacle which would present much less mechanical resistance than the imposed glass filaments. For this purpose glass spheres about 70 μ in diameter, laying at the bottom, were used. Amoebae were moving free between the spheres and frequently came in contact with them by the front of advancing pseudopod or by the lateral body side. Such conditions differ essentially from these which are involved by the glass

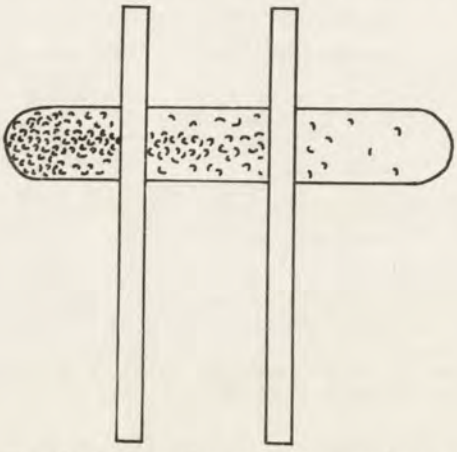

Fig. 6. Explanation of the experiment with glass filaments, as suggested by the theory of membrane folding. The membrane fails to unfold between the points supporting the load

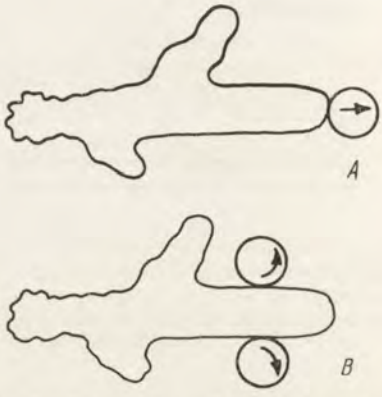

Fig. 7. Behaviour of the glass balls on the contact with the surface of an amoeba. A. Pushing of the sphere by the tip of an advancing pseudopod. B. Rotating of the spheres by the lateral surface of amoeba

fibres, because the sphere is not a load placed upon amoeba but it lies on the substrate touching it in a single point as in a ball bearing. This enables a rather easy shifting or rotating the sphere by the animal.

As schematically represented in the Fig. 7 A, when an advancing pseudopod meets a sphere on its way, a simple shifting occurs. Amoeba pushes the sphere straight ahead, as long as it hits just upon the axis of movement of the pseudopod. Of course, this result coincides well with the majority of theories: the theory of stationary condition of cell membrane, that of membrane flow, and with the present hypothesis of membrane folding. On the

other hand it gives an evidence against the theory of membrane rolling, on the ground of which keeping the ball on the tip should be expected impossible. It would be removed off the front of amoeba sideways, or at least rotate in the vertical plane. The experiment proves, on the contrary, that the movements of cell surface are essentially symmetrical in respect to the axis of locomotion of the whole cell (or that the membrane does not move at all).

If the sphere slips away from the tip of pseudopod (a slight change in the direction of movement is sufficient to involve this) and slides down the side, or an animal comes in contact with a sphere at once by the lateral surface, another phenomenon occurs which is presented in the Fig. 7 B. The sphere starts to rotate, mainly in the horizontal plane, and the direction of its revolving coincides with the direction of the locomotion of amoeba. In the opinion of present authors this result excludes the idea that the membrane is stationary but proves that it moves in the same direction as the whole cell does.

Occasionally the glass spheres lying on the side of amoeba may remain in rest. However, the examination of such a case reveals that amoeba embraces partly the sphere by forming corresponding convexities or even distinct pseudopods. That means that such a sphere is no longer a loose body only touching the amoeba but it becomes merely a new site of adhesion. Animal may adhere to the ball in similar manner as it adheres to the substrate, and therefore in such a case the cell surface adhering to the sphere remains motionless as well as that adhering to the bottom.

Nevertheless, the most typical situation is a simple contact between amoeba and a sphere, which does not impede the free unfolding and shifting forward of the cell surface, and results in a corresponding rotation of the sphere.

Commonly known is the experiment of Goldacre 1961 with oil drops "attached" to the cell membrane. The oil drop becomes firmly fixed upon the cell membrane if the oil is injected from inside, i.e. against the inner side of the membrane (cf. Pl. XII 42—43). The following proposals could be made here as to the mechanism of this "attachment". Oil joins the bimolecular lipid layer of the unit membrane and forms the same phase with it, that is to say there are no interfaces between the oil drop and the endogenous lipids of the cell membrane (see Fig. 8). This should mean that the oil drop is not simply attached to the membrane but it becomes in fact its constituent part.

The first oil drop injected into the cell and build into the the cell membrane becomes the leading cap of amoeba. It persists on the tip of advancing pseudopod and is pushed ahead, like the glass ball used in the former experiment (cf. Pl. XII 45—47). In this respect the finding of Goldacre 1961 is entirely confirmed by the ours. In this case also the result may be explained by every theory suggesting any kind of symmetrical shifting of the cell surface or its stationary condition, being incompatible only with the theory of rolling.

The second (smaller) oil drop — as described by Goldacre 1961 and confirmed in the present experiments — may join the cell membrane laterally, and in such a case it remains stationary, fails to move forwards with the cell, and finally is squeezed off from the cell surface in the tail region. This result is considered by Goldacre as a crucial evidence of the sta-

tionary condition of the cell membrane during amoeboid movement. However, it should be rather amazing to expect any transport of the relatively big oil drop by the shift of extremely thin molecular layers constituting the cell membrane.

Another explanation may be put forward basing on the view, presented above, that the lipids of the oil and the lipids of the unit membrane link into one entity. The oil drop in aqueous phase is composed on its boundary (the oil-water interface) of monomolecular layer of polarised molecules orien-

Fig. 8. Scheme explaining the postulated mechanism of the "attachment" of the oil drop to the cell membrane. Only the bimolecular lipid layer of the unit membrane is represented by the diagram. The layer of oriented molecules is also represented on the boundary of the oil drop, as joining the layer of the native membrane lipids

ted by their hydrocarbon ends inside, i.e. in the same manner as they are oriented in the bimolecular lipid layer of the unit membrane. It seems therefore reasonable to postulate, that the outer monomolecular lipid layer of cell membrane becomes continuous with the monomolecular lipid layer on the boundary between the oil drop and the external medium. In the same way the inner lipid layer of the cell membrane forms probably one entity with the layer of polarised molecules on the boundary of oil and cytoplasm. A scheme of this molecular configuration is presented in the Fig. 8. If this was true, it should be postulated that only the molecular layers covering the oil drop would slide on the surface and would move with the general shifts of the cell membrane, but the bulk of oil could remain at rest.

The final detachment of the oil drop from the tail surface may be explained as suggested by the Fig. 9. The oil drop in water tends to assume a spherical shape. This is not possible when it forms a part of the cell membrane in the anterior section of amoeba, since the cell membrane is stretched there. The oil may assume only a hemispherical shape, as it is in fact observed as well by Goldacre 1961 as by us. When the oil becomes the element of the membrane coating the tail, the situation changes because the membrane is folded in this region and sufficiently loose to allow the oil to assume gradually a spherical shape. Finally it forms a regular sphere, linked with the cell by a single fold of membrane only ("small filament, or neck of membrane" — as referred by Goldacre 1961), and at last it detaches completely.

It may be stated in general that the fate of oil drops seems to be explained by the membrane folding theory and the postulated behaviour of the monomolecular lipid layers. On the other hand, the stationary condition of the big oil drop bound into the lateral membrane could be hardly considered as evidence of the stationary condition of subtle molecular lipid layers constituting the boundary of the cell.

An increase in viscosity of the external medium may also be considered as a special kind of mechanical obstacle impeding the movement of amoeba. However, this factor has not been examined up to now. In the present experiments amoebae were placed with very small quantity of Chalkley medium on a depression slide filled with 2% methyl cellulose solution. After

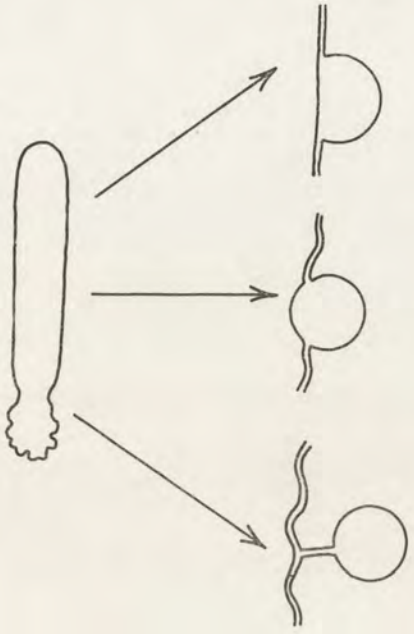

Fig. 9. Diagram representing the relation between the degree of membrane folding and the shape assumed by a laterally located oil drop. High convolution of the membrane in the tail region allows the final detachment of the oil

mixing the fluids the drop was left open to evaporate gradually under continuous microscopic control. The behaviour of polypodial and monopodial forms was different under such conditions.

In the polypodial amoebae both the locomotion of the whole cell and the internal cytoplasmic streaming gradually slow down. Finally the locomotion and streaming cease simultaneously, and the cells appear like entirely paralysed. This occurs at the viscosity level which appears not to be extremely high, because the ciliates supplied as food (*Tetrahymena* and *Colpidium*) are still swimming around the immobilized amoebae. Dilution of the methyl cellulose results in an immediate recovery of the locomotion as well as of the intracellular streaming.

In the monopodial forms the locomotion becomes also entirely inhibited following the increase in viscosity, however the cytoplasmic streaming persists in a modified form: the so called "plasmagel" which is stationary under normal conditions, starts to flow backwards at the same rate as the "plasmasol" flows forwards, i.e. a "fountain" appears and covers all the cell body.

Despite the apparent difference, it should be stressed that as well the cease of streaming in the polypodial amoebae as the full fountain in the monopodial ones bring about the same result: the cell material is no longer transported effectively forwards. Therefore, it may be stated in general that no component of amoeboid movement may be preserved if the cell surface is artificially made stationary in fact.

The last kind of mechanical obstacle the effect of which was observed in the present research, were other amoebae met on the way of one another. The fact put forward by different authors as an argument in favour of the stationary condition of the cell surface was that amoebae may crawl one upon another in different directions without affecting mutually their movement. This argument seems to be hardly acceptable for two reasons: no quantitative data are available and it is not known whether the movement of two overcrawling amoebae is in fact so effective in opposite directions as in the conforming ones; on the other hand, the cell surface of both amoebae may become stationary in the area of contact as it probably does at the

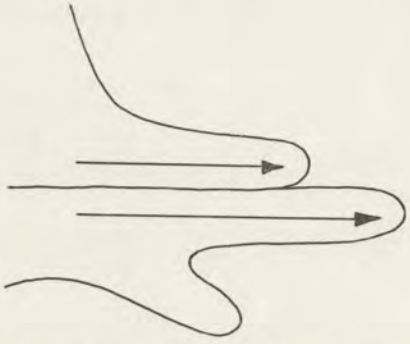

Fig. 10. Coincidence of the directions of movements in the pseudopods of two amoebae which have met one another

points of adhesion and at the points supporting a load (e.g. the glass filaments).

The picture seems to be more clear when the behaviour of two amoebae which are touching one another by sides is taken into account. The results of some tens of observations are summarized in the Fig. 10, which shows that an extending pseudopod which meets the body of another amoeba on its way usually turns in the direction consistent with the movement of the encountered animal (only in a separate cases the result was different or rather unclear). This is just what was to be expected if the cell membrane on the side of encountered amoeba is gradually unfolded and shifts forwards.

Movement of particles attached to the cell surface

The classical type of experiment concerning the behaviour of cell membrane during locomotion of amoebae is the analysis of movement of small granules (most commonly carmin particles) adhering to the cell surface. The observation of carmin particles moving forwards on the upper surface of different amoebae, with a speed exceeding the rate of locomotion of the whole cell, led J e n n i n g s 1904 and M a s t 1926 to formulate the theory of mem-

brane rolling. In recent time, these observations were confirmed, and the theory of rolling was supported by Griffin and Allen 1959 and by Abé 1962. The criticism of experiments with carmin particles expressed by Goldacre 1961 will be discussed in the next chapter of this paper.

The following experiments were performed to reconciliate different data on the behaviour of particles attached to the cell surface of amoeba.

Instead of carmin particles, the crystals of calcium oxalate were applied. The most minute from them, measuring a few microns, move forwards indeed over the surface of an advancing pseudopod. The crystals of Ca-oxalate rotate the plane of polarized light which is not characteristic of the crystallic inclusions present in the cytoplasm of amoeba. This allows to use the polarization microscope to observe simultaneously the behaviour of crystals adhering both to the upper and the lower surface of amoeba. By means of this technique it has been revealed that, in most cases, the small crystals fail to move on the upper surface forwards and on the lower one back-

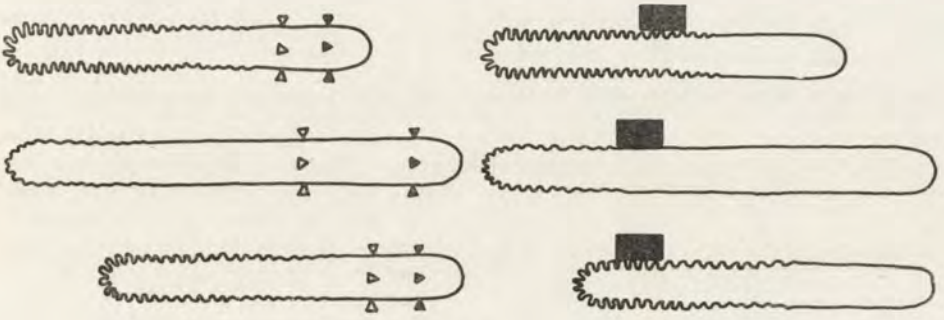

Fig. 11. Diagrammatic representation of the postulated mechanism of the behaviour of small particles adhering to the surface, which are symmetrically transported forwards in relation to the ground. Compare the corresponding stages of folding and unfolding in the Fig. 2

Fig. 12. Diagram explaining the mechanism of the gradual accumulation in the tail region of the big particles adhering to the cell surface. Compare the corresponding stages of folding and unfolding in the Fig. 2

wards, as it has been postulated by the theory of rolling, but they may proceed towards the tip symmetrically from all sides. This result agrees well with the postulations of the present theory of membrane folding and unfolding.

Another point of the present concept, concerning the stationary condition of the cell surface in the regions adhering to (or supporting) solid bodies, has been also confirmed in the following way. Amoebae were placed in a drop of medium on the microscopic slide which surface was previously covered by crystals of calcium oxalate. Under such conditions photomicrographs were taken in the dark field illumination with the exposure time of 30 sec. The camera was focussed on the crystals laying at the bottom, not on the amoeba which appears as a bright cloud on the top of them (Pl. VI 13). Despite the prolonged time of exposure all crystals appear as sharp spots, none of them presents a stripe. This proves that the animal

passes over the crystals covering the substrate without shifting them i.e. the cell surface adhering to the bottom is at rest.

The essential mechanism of the behaviour of small crystals adhering to the cell surface of amoebae, which follows from the concept of membrane unfolding, is represented by the Fig. 11. As it is clearly seen, the adhering particle should move really in relation to the ground and rather apparently in relation to the cell. This scheme may be supported by the following observations. 1. The symmetrical shifting of particles forwards is in average rather apparent when the cell (not the ground) is taken as point of reference. The simple evidence of this fact is that in spite of the displacement of particles forwards they do not accumulate at the tip of pseudopod. 2. The distance between two particles laying one behind another along the axis of the same pseudopod oscillates rhytmically, as it is postulated by the scheme of folding and unfolding. 3. The distance between two particles adhering to two neighbour pseudopods (in the anterior part of animal) increases. This last phenomenon has been also described in details by Jahn 1964 (cf. his Fig. 1) and considered by him as evidence of the formation of new membrane, however it fulfills also entirely the postulation of the present concept that the membrane unfolds between two advancing pseudopods[1].

The only fact which is apparently controversive is that some particles may move really in relation to the cell and approach the tip of the pseudopod. Nevertheless on the other hand, it may be seen that simultaneously other particles on such pseudopod move slower than the cell or even remain stationary. Therefore this phenomenon confirms only the view presented above that the rate of unfolding and shifting the cell membrane is not necessary uniform around the whole pseudopod, because the folded membrane does not behave as a rigid body.

It is a striking paradox that the behaviour of adhering particles has two contradictory aspects which are emphasized by both opposite theoretical schools. Namely, in spite of the apparent forward movement of the adhering material, it finally accumulates not at the tip of pseudopods but it forms clumps in the tail of amoebae. Of course, this second phenomenon is pointed out by the authors postulating the stationary condition of the cell surface. Chapman-Andresen 1964 reported the accumulation of polystyrene spheres on the tail of *Amoeba proteus*, and Shaffer 1963 found a similar accumulation of clumps of food residues on the tail of *Polysphondylium violaceum* (amoeboid stage).

In the present study the formation of clumps of particles on the amoeba's tail was clearly seen in all experiments with the crystals of calcium oxalate. Nevertheless, it was stated that only the relatively bigger crystals accumulate on the tail. This observation allows to explain the origin of the clumps, as this is presented in the Fig. 12. A big particle which adheres to numerous folds of the membrane cannot follow precisely the unfolding and folding of the cell membrane, and — as a consequence — its forward movement is

[1] The mechanism of folding and unfolding, as suggested in this study, seems to agree in some extent also with the behaviour of small particles adhering to the surface of "pseudodigits" of the amoeboid stage of *Polysphondylium violaceum* (Shaffer 1965 a), and of the surface markers adhering to the "grex" of *Dictyostelium discoideum* (Shaffer 1965 b).

retarded in respect to the movement of the whole cell. Finally such particles accumulate on the tail[2].

It may be stated in general that the theory of membrane rolling (and that of membrane flow) explains shifting forwards of particles adhering to the surface of amoeba but it postpones their final accumulation on its tail. On the contrary, the theory of stationary condition of cell membrane explains the formation of the clump in the posterior end of amoeba but it is essentially inconsistent with the forward movement of the granules. The present concept seems to explain both apparently contradictory phenomena assuming that all the particles move forwards with the cell, following the folding and unfolding of its surface, but the bigger ones are retarded and finally accumulate on the tail.

Streamings promoted by amoebae in viscous medium

Some new aspects of behaviour of the cell surface of moving amoebae were revealed by photomicrographic registration of streamings arising around the animal in the medium. The technique presents in fact a modification of the method introduced earlier by one of the present authors (Grębecki 1961) for photo-registration of streamings promoted by the cilia of *Paramecium*. The procedure applied for the study of amoebae was the following.

A slightly viscous methyl cellulose solution (0.2—0.5%) was prepared and the minute crystals of calcium oxalate, measuring a few microns, were suspended in it. Thereafter, equal volumes of this viscous suspension and of the culture medium with amoebae were mixed together. The increase in the viscosity of medium plays in the experiment two separate roles. Firstly, it prevents the sedimentation of suspended crystals. Moreover, it produces the "amplification" of every effect of pulling and pushing the medium by the cell moving in it. It should be stressed on the other hand, that the experimental conditions in question did not result in suspending amoebae themselves, but the animals continued to move being attached to the substrate.

A drop of the mixture described above was then transferred upon a microscopic slide and covered with a cover-slip, supported by some glass balls (150 μ in diameter) in order to prevent squashing the cells. The preparation was mounted under a low power microscope equiped with a dark-field condensor, and left for a few minutes until any passive shift of the viscous fluid with crystals suspended in it ceased. Thereafter, the photomicrographs were taken on a high speed negative material[3] with the exposure time varying from 15 to 45 sec. Under such light conditions each crystal of calcium oxalate

[2] Eventual explanation of the formation of clump might be also suggested in agreement with the theory of rolling. It should be postulated, in this case, that the accumulated particles are taken by amoeba from the substrate. However, it was stated in the present experiments that the clump is never formed if the amoeba itself is free from particles and only the bottom is covered by them.

[3] When a low speed film is used rather the movement of the intracellular crystallic inclusions is recorded, i.e. the so called "pictograms" of the intracellular streaming are obtained, as in the technique of Rinaldi and Jahn 1963.

appears as a bright spot and every shift of the crystal, occurring during the exposure, is registered by the camera in the form of a distinct stripe. The stripes formed as result of displacement of numerous crystals surrounding the body of amoeba provide a general picture of streamings evoked by it in the medium (cf. Pl. I—VII).

The picture presented by the Pl. I 1 allows to evaluate the reliability of records provided by the method described above. Two animals are simultaneously present in the field. All the crystals disposed on the margins of the picture present simple spots, i.e. they were motionless during exposure. This means that the streamings arising around the amoebae are in fact induced by them, and they do not represent any general passive flow of the liquid. The second evidence for the active character of the phenomenon under question comes from this fact that two amoebae are not surrounded both by a common streaming, but on the contrary — each of them forms its proper system of streamings around it.

The general character of streamings arising around a polypodial amoeba is shown by the Pl. II 2—3. The most striking aspect of this system of flows is the fact that they commonly assume a pattern of whirles. The whirles arise always between the advancing pseudopods and the withdrawing ones or between the advancing pseudopods and the amoeba's tail. Near the cell surface the external fluid flows forwards, i.e. in the same direction in which the animal moves and in which flows its intracellular cytoplasmic streaming. Every change in the intracellular streaming is immediately followed by a corresponding change in the extracellular flow. At some distance from the cell the viscous fluid comes back, forming in this way a more or less broadly closed circuit. This recurrent streaming, considerably distant from the cell, seems to be passive or rather a secondary one, that is to say it represents only the arm closing the full cycle of whirle, conforming the principles of hydrodynamics.

As it is seen from the general picture given in the Pl. II 2—3, the system of whirles surrounding an amoeba is in most cases essentially symmetrical. The viscous fluid is pushed and pulled by the amoeba evenly at both sides seen in the optical section, i.e. in the plane of the photomicrograph, and probably — evenly in all other planes.

There are some exceptions, when the system of whirles is not symmetrical, but these are fully explained by the character of movement manifested by the amoeba during registration. Pl. III 4 and 5 give two successive records of the same animal taken at interval of 30 sec. In the first photograph the whirle is much better pronounced in the left upper corner, i.e. at the left of amoeba, than on its opposite side. 30 sec. later (the second photograph) the inverse picture arose: the distinct whirle has been formed at the right side of amoeba while the left one turned to be scarcely visible. The reasons of this change are rather clear. During the first registration, the leading tip of amoeba was constituted by the large pseudopod extended from the left side of the body (labelled "*a*"), but at the moment of second registration, the initiative has been already taken by the small pseudopod protruding from the right body side (labelled "*b*"). This means that the distinctness of the whirles depends on the character of locomotion, i.e. the viscous medium is pushed and pulled more effectively at this side at which the processes of folding and unfolding of the cell surface are actually more intense.

Observations of streamings surrounding the advancing tip and the tail region of amoeba lead to the same conclusions. The shifts of the viscous medium at both sides of an extending pseudopod (Pl. IV 6), as well as at both sides of the tail (Pl. V 8), are almost in all cases symmetrical. Asymmetry, however, may arise when the advancing pseudopod, for example, changes the direction of its movement (Pl. IV 7), or when the tail undergoes a lateral contraction (Pl. V 9), i.e. when an asymmetry of the membrane unfolding should also be expected.

Another feature of the streamings observed in the medium, which should be pointed out in relation to the theory of membrane folding, is the fact that — when they are symmetrical — their speed seems never to exceed the speed of locomotion of the whole cell, which supports the view that they result from pushing and pulling the viscous medium by the shifts of the cell surface.

Finally, it should be stressed that the postulated rhytmicity of membrane folding and unfolding is also reflected in the character of streamings arising in the medium. It may be seen in all registered pictures that the paths followed by individual crystals are not straight lines but rather indicate some kind of saltatory movement. This character of movement of the crystals is shown in high magnification on the Pl. V 10. The exact identicity of paths described by all neighbour crystals proves that the observed vibration is not due to the Brownian movement, but that it reflects the rhytmicity of pulling the medium by amoeba itself. This subject will be treated in details elsewhere.

Two last records (Pl. VI 11—12) concern the monopodial forms. In such amoebae the general picture of extracellular streaming is essentially the same as in the polypodial ones. Often the picture is slightly asymmetric which could produce the impression of some kind of membrane rolling. This effect is strongly co-related to the asymmetry of movement: a monopodial amoeba usually manifests periodical deviations of direction of the internal streaming of cytoplasm accompanied by an oblique position of the hyaline cap or even such an asymmetry may persist for longer time and result in a permanent bending of the body. This means that in monopodial amoebae an eventual asymmetry of the streamings promoted in the medium reflects also the facultative asymmetry of unfolding the cell membrane.

It may be stated in general that the photographical analysis indicates that the streamings arising in the viscous medium around the moving amoebae result from pushing and pulling the external medium by the cell surface which shifts together with the whole cell, by simultaneous folding and unfolding. The pictures provided by the photo-registration technique are essentially inconsistent with the theory of stationary condition of the cell membrane, which could explain only some shifts of medium in front of the cell and behind it, but not the conspicuous whirles formed all along the lateral surface. In most cases, they are also inconsistent with the theory of membrane rolling, because the whirles are usually symmetrical, and their occasionnal asymmetry which may temporarily appear, is purely facultative and co-related with the asymmetry of movement[4].

[4] Sometimes an illusion of rolling may also arise when an inactive pseudopod is observed, which behaves as a simple lateral convexity of the body, i.e. the shifts of the unfolding membrane probably may pass over it.

Finally, the experiments with crystals suspended in methyl cellulose solution allow to discuss the views of Goldacre 1961 as to the movement of carmin particles and other granules. Goldacre postulates that their behaviour does not reflect any shifts of the cell surface but is, in fact, an electrophoretic movement in a field of potential created by the cell itself.[5]

There are two reasons for disputing such an interpretation in the light of present experiments: 1. If the concept of Goldacre was true, the crystals should move from the contracting region of the body towards the tip of the pseudopod throughout all the field, that is to say a spindle should arise, not a whirle. 2. The increase in the viscosity of medium should impede the movement of crystals, and not "amplificate" this effect.

Plasma-membrane ratio

It seems reasonable to postulate, on the ground of the present concept of membrane folding and unfolding, that not only the movement of amoeba but also its shape should strongly depend on the ammount of "excessive" surface material coating the cell body.

The relation between the volume of cytoplasm and the surface area of cell membrane in the same cell will be called in the following pages: plasma-membrane ratio, the term being formed analogically to the commonly known notion of the plasma-nuclear ratio. If the two postulations of the present theory were true, namely that the cell membrane is a relatively permanent structure and that it is not extensible mechanically, a further anticipation could be made: it should be expected that the cell shape is controlled by the plasma-membrane ratio. Of course, the plasma-membrane ratio is the highest in the spherical cells with a smooth surface and it gradually decreases as the cell shape becomes more complicated.

The cell of *Amoeba proteus* which may display different degrees of complication of the polypodial form, or assume a monopodial form, or even a spherical shape, provides an excellent material for such a study. Polypodial specimens of amoebae (Pl. VII 14) as well as the monopodial ones (Pl. VII 15) are both commonly encountered together in the same cultures. The reasons of this difference in shape are unknown up to now. Spherical or discoidal forms were obtaind by some authors under experimental conditions (Goldacre 1958, Czarska and Grębecki 1965, Landau 1965, Seravin 1966). According to the present hypothesis, these forms could all be arranged in the series: polypodial→monopodial→spherical, which would correspond to the increasing plasma-membrane ratio. In other words, it should be possible to obtain artificially any form of amoeba by altering this proportion. Two opposite modes of controlling this ratio may be conceived, namely affecting the volume of the cell content, or reducing the amount of the surface material. Both ways are accessible in the experimental approach.

[5] This interpretation has been already criticised by Shaffer 1963; this author indicates that Goldacre compares the forward movement of negatively charged particles with the passive accumulation in the tail of positive ones, whereas in fact the granules of both anionic and cationic exchange resins accumulate on the tail, like any other material does (these arguments are in full accordance with the present interpretation as presented in the p. 217).

Effect of changes in the cytoplasm volume

The simplest way to reduce the volume of cell content is to induce an outflow of water from the cell by exposing it to a hypertonic medium. Amoebae were transferred from the normal Chalkley medium into the concentrated sucrose solutions varying from 50 up to 150 mM. As this is diagrammatically demonstrated by the Fig. 13 A, the specimens which were perfectly monopodial produce immediately additional pseudopods and transform into polypodial ones. Other cells which were initially polypodial in the original medium (Fig. 13 B) appear even more polypodial, that is to say their pseudopods become distinctly longer and thinner, more numerous and distributed at random. After being passaged back to the normal Chalkley medium, the cells recover their former shape (the return to a typical monopodial form seems to

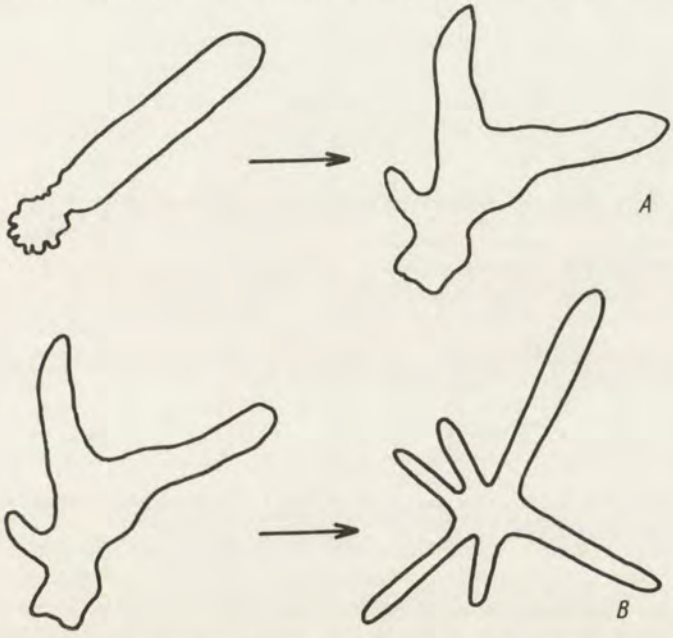

Fig. 13. Transformations of shape manifested by the amoebae exposed to the 100 mM sucrose solution. A. Transformation of a monopodial specimen. B. Transformation of a typical polypodial specimen

be slightly more difficult than a simple reducing of number and length of pseudopods).

Analogous experiments were performed in the solutions of NaCl (25—75 mM). The results proved to be almost identical with that in case of sucrose. The only difference consisted in the fact that in the latter experiment a self-regulation of the cell shape took place. This seems to depend on the properties of the cell membrane which probably is in amoebae (as in other cells) impermeable for sucrose but permeable for sodium ions.

Since reducing of cell content by leakage of water in hypertonic media gave the expected results, it could be also anticipated that — inversely — in-

creasing of the cytoplasm volume by imbibing water in hypotonic media should involve an attenuation of the polypodial character of amoebae. In order to verify this supposition, the polypodial amoebae were transferred from the Chalkley culture medium into distilled water. Really, the length and the number of pseudopods seemed to be distinctly reduced, however no true monopodial forms were obtained, that is to say the result was positive but rather incomplete. Of course, this is not surprising at all because the ionic strength of the normal Chalkley medium is very low ($I = 0.0015$) and, therefore, only a slight hypotonic effect of distilled water could be expected.

To overcome this difficulty, in another series of experiments, amoebae before the transfer to distilled water, were adapted to the media of higher ionic strength. The Chalkley solutions of concentrations 2×, 4×, 8×, and 16× higher than in the normal culture medium have been applied. The adaptation procedure was gradual, i.e. the culture was exposed to the concentration twice higher from day to day. Before changing the medium a number of cells were sampled and two photographical pictures of them were taken: the first immediately in the adaptation medium, and the second one after exposure to distilled water.

The photomicrographs of the control samples demonstrate that after raising the concentration of the Chalkley medium from 2× to 4× (Pl. VIII 16 and 18) amoebae become more polypodial, in the concentration 8× they assume the shape of pinocytotic rosettes (Pl. VIII 20), and in the last medium (16×) some cells retain the pinocytotic form (Pl. VIII 22a) but the others break up and only their "ghosts" remain (Pl. VIII 22b).

The effect of hypotony in such pre-adapted cells is much more distinct than it was observed in normal cultures. Amoebae transferred into distilled water from the Chalkley medium concentrated 2× clearly reduce the number of pseudopods (Pl. VIII 17), transferred from the medium concentrated 4× become nearly monopodial (Pl. VIII 19), those which were passaged from the concentration 8× are monopodial almost exactly (Pl. VIII 21), and finally those which had resisted the concentration 16× may assume even the form of smooth spheres (Pl. VIII 23).

It may be stated in general, that the experiments with the hypertonic and hypotonic media support the present view that the cell shape in amoeba depends on the amount of the "excessive" surface material, i.e. on the plasma-membrane ratio. When this ratio decreases on account of a decrease of the cell volume (hypertonic media) the shape of amoeba evolves in the direction of gradually more polypodial form. Inversely, the increase of the plasma-membrane ratio involved by an increase in the cytoplasm volume (hypotonic media), is followed by the transformations of shape leading to the monopodial or even spherical forms.

The aim of the subsequent work was to simplify the conditions of experiment, that is to say to affect the cytoplasm volume in a more direct way. The first step was to exclude the adaptation of amoebae to the concentrated Chalkley medium. Besides adaptation, there exists a simpler way of raising the ionic strength inside the cell, namely the microinjection of a concentrated electrolyte solution. Photographical record of one of such experiments is presented by the Pl. IX.

A distinctly polypodial specimen has been choosen for the microinjection (its shape as seen in the Pl. IX 24 is due to the contraction involved by the

mechanical shock — insertion of the micropipette inside the cell). The solution of NaCl 100 mM has been injected. Amoeba starts to move just in the moment of injection (Pl. IX 25) and then shifts soon away from the instrument (Pl. IX 26). At the beginning it remains still polypodial (Pl. IX 27). Thereafter, the uptake of water promoted by the high intracellular ionic strength involves an increase of the cell volume, and — as a result — of the plasma-membrane ratio. This brings about the corresponding transformation of shape, i.e. assuming a nearly monopodial form, as it is shown by the picture taken 5 min. after the microinjection (Pl. IX 28). The sodium ions probably may pass through the cell membrane of amoeba, and the regulation of ion distribution inside and outside the cell should result in the recovery of the initial plasma-membrane ratio, and—as a consequence—in resuming the former shape. In fact, about 30 min. later the amoeba turned to be polypodial again (Pl. IX 29).

In other experiments of this series the results were quite similar. The effect may be more or less pronounced and its manifestation may slightly vary in time, depending only on the concentration and on amount of injected solution of sodium chloride.

A still simpler way of increasing the plasma-membrane ratio by affecting the volume of the cell content is the microinjection of water or rather of external medium. This kind of experiment is shown by the Pl. X.

The micropipette filled with normal Chalkley medium is inserted into a polypodial amoeba (Pl. X 30). During the abundant injection the cell blows up as a balloon (Pl. X 31). Finally it assumes a perfectly monopodial form and moves away from the micropipette, as demonstrated by the Pl. X 32. Of course, such monopodial cell cannot keep its shape longer than for a few minuts, because the injected water leaks very easy out of the cell.

It is also possible, to inflate the amoeba to such a degree as to obtain a spherical form, by more abundant injection of the Chalkley medium (Pl. X 33). In this respect it should be pointed out that such a sphere is incapable to further swelling, but is disrupted by any excessive amount of the injected fluid (Pl. X 34—35). This last result confirms the view, put forward by Wolpert, Thompson and O'Neill 1964 and supported in the present study, that the cell membrane of amoeba is incapable of an elastic stretch without any moleculur re-arrangement.

In general, it seems possible to conclude that every change in the cell volume, induced as well by the osmotic effects as by microinjections, affects the plasma-membrane ratio, and — as a result — it controls the shape of amoeba. Consequently, the cell membrane of amoeba should be considered to be a relatively permanent structure.

Effect of changes in the membrane area

As pointed out above, the second imaginable way of altering the plasma-membrane ratio is to change the amount of the surface material covering the cell. There exists a simple means, easy practicable in the experiment, to produce such a change: that is to induce pinocytosis which results in the ingestion of a considerable quantity of the external membrane material and in its transfer into the cell inside. As a result of such a decrease in the external membrane area, an increase of the plasma-membrane ratio should be expected, and this should be followed by a tendency of polypodial amoebae to produce the monopodial or even spherical forms

In fact, such an experiment has been already described in the foregoing chapter (cf. p. 222), namely the monopodial and spherical forms were obtained in distilled water from amoebae which manifested pinocytosis in the Chalkley medium concentrated 8× or 16× (Pl. VIII 20—23). Nevertheless, this observation seems not to be quite satisfactory because two different factors were simultaneously involved in the experimental conditions: besides the decrease of the amount of the surface material by pinocytosis, as well an increase in the cytoplasm volume by the osmotic effects took place.

In order to ascertain unambiguous experimental conditions, in further study the pinocytosis was induced by the action of alcian blue which concentration (1 : 10 000) is too low to exert any osmotic effect. A number of polypodial amoebae (see the control picture — Pl. XI 36) were exposed to the action of the dye, and about 15 min. later, when the animals still kept the shape of rosette but had already ingested an important portion of the coloured membrane, a drop of this preparation was put in the middle of a glass plate covered by a layer of the normal Chalkley medium. The plate was mounted on the microscopic stage.

As a result of the dilution of alcian blue, the amoebae stop pinocytosis and their locomotion gradually reappears. Slowly the animals leave the area contaminated by the dye and enter the pure Chalkley medium. On their way they undergo significant transformations of shape. Some of them could be eventually called polypodial but their pseudopods are very short, thick, and not numerous (Pl. XI 38); moreover, such forms are rather few. The most commonly produced form is an exactly monopodial amoeba which lacks only the hyaline cap (Pl. XI 39). Nevertheless, a very significant number of specimens (up to 15%) assume the perfectly monopodial form bearing the typical hyaline cap (Pl. XI 40). Finally, some animals occur which assume even the spherical shape (Pl. XI 41).

It may be stated that the reduction of the surface material by pinocytosis increases the plasma-membrane ratio which really results in the transformation of shape in amoeba, leading towards the monopodial or spherical form. It might be expected furthermore, that the monopodial amoeba formed by a decrease of the membrane area should be considerably smaller than the monopodial specimen which arose owing to an increase in the cytoplasm volume. As a matter of fact, such a difference in size is very distinct when a typical monopodial cell formed by pinocytosis (Pl. XI 40) is compared with a typical monopodial specimen taken from the original culture (Pl. XI 37).

Another means of affecting the plasma-membrane ratio, and inducing the expected shape transformations in amoeba, is provided by the experiment of Goldacre 1961 (repeated in this study — see p. 211) with oil drops injected inside the cell against the inner surface of the membrane. It could be expected that, if the oil drop joins the native lipids of the unit membrane and forms a common phase with them (cf. Fig. 8) the surface tension at the oil water interface should tend to "suck" the bimolecular layer of membrane lipids into the oil drop[6]. The expected course of the phenomenon is represented diagrammatically by the Fig. 14. At the beginning, since the membrane

[6] Such a "sucking" may be demonstrated by a simple model. The mixture of the paraffin oil with xylene forms a film on the surface of water, and this film may be sucked into a drop of pure oil put on it.

is considerably convoluted, its sucking into the oil drop should result in stretching and unfolding (Fig. 14 A—B). When no more free membrane folds are present, the further sucking should transform the whole mass into a sphere (Fig. 14 C). Finally, if the sucking force of the surface tension exceeds the mechanical resistance of the bimolecular lipid layer, the membrane could burst at the pole opposite to the oil drop (Fig. 14 D).

In other words, it is assumed that amoeba should undergo the shape transformations leading to the monopodial and spherical forms for two reasons: 1. because the amount of the surface material becomes reduced by

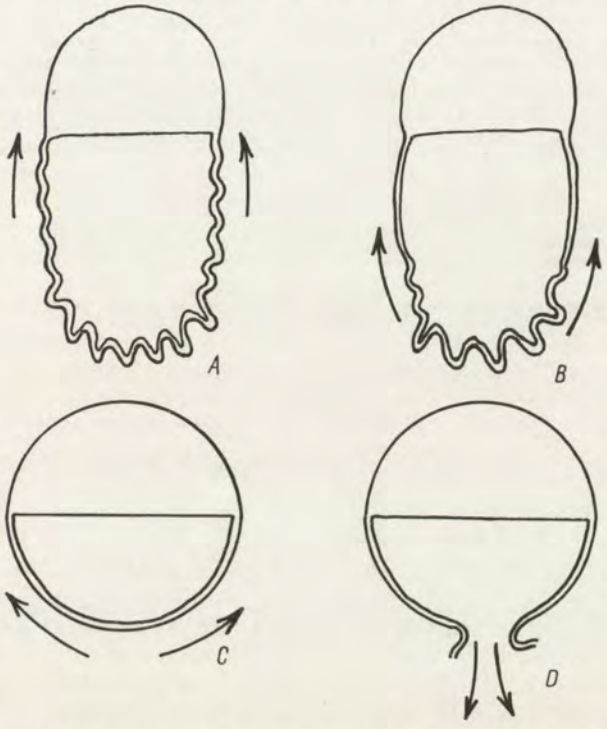

Fig. 14. Diagrammatic representation of the presumable behaviour of the cell with a big oil drop joining its membrane. A. Initial stage. B. Intermediate stage of unfolding the cell membrane by "sucking" it into the oil drop. C. Stage of the maximal stretching of the membrane. D. Breaking up of the cell membrane at the pole opposite to the position of the oil

"sucking" it into the oil drop (i.e. because the plasma-membrane ratio increases), 2. because the oil and the endogenous membrane lipids form a common phase, i.e. a joint lipid body which carries to reduce maximally the area of its contact with the water phase, and tends to become a sphere. It seems obvious that both reasons are in the physical terms the same.

Of course, the scheme presented above presumes a quite passive behaviour of the cell, but it should be kept in mind that actually the amoeba actively contracts and folds its membrane in the tail, opposing in this way the suck-

ing force of the oil drop. Nevertheless, it has been proved in the experiment that after a sufficiently abundant injection of oil the phenomenon runs almost strictly as it has been expected. Photographical record of one of such microinjections is shown by the Pl. XII and XIII.

The micropipette filled with paraffin oil has been inserted into a polypodial specimen (Pl. XII 42). The injected oil drop joins the cell membrane and starts to exert its influence immediately, just in the moment of injection. This is demonstrated by the picture taken in the course of injection (Pl. XII 43), in which as well the leading cap of amoeba already formed by the oil, as a still persisting oil channel between the drop and the tip of the pipette, are seen simultaneously.

Soon after microinjection, the anterior part of amoeba forms a bulb close to the oil cap, and the posterior part manifests still a high degree of folding of the cell surface (Pl. XII 44). This stage corresponds strictly to the scheme anticipated in the Fig. 14 B, that means it demonstrates that the cell membrane is stretched and unfolded by the oil drop, and the stock of the convoluted membrane persists on the opposite pole, in the amoeba's tail. As it is shown by three following pictures (Pl. XII 45—47), further sucking of the membrane into the oil drop results probably in a more uniform distribution of membrane folds, since the anterior part of amoeba following the oil cap seems to be less stretched, and the posterior one less convoluted. The shape assumed by the cell at this stage corresponds precisely to the typical monopodial form of amoeba. Such monopodial amoebae with oil caps may preserve their shape for a long time. It should be concluded that, up to this phase, the experiment confirms the observations of Goldacre 1961 but, on the other hand, it supports also their interpretation put forward in this study.

Subsequent fate of monopodial amoebae produced by an abundant oil injection has not been reported by former authors. As this is shown by the following pictures (Pl. XII 48—49), the monopodial amoeba gradually becomes slightly thicker and shortens very distinctly. Simultaneously, the oil drop clearly seems to increase in size. These symptoms indicate that the whole mass tends already to assume the spherical shape. The next step is a further increase of the sphere, only with the amoeba's tail remaining outside (Pl. XII 50 and XIII 51). The tail surface appears at this stage still convoluted which supports our view that the active posterior contraction of amoeba counteracts the sucking force of the oil drop.

Finally, even the tail disappears and the whole cell joins oil to form a common spherical mass. The sphere is initially not quite perfect (Pl. XIII 52—53). Thereafter it assumes a regular shape (Pl. XIII 54) which indicates that no further reduction of surface of the cell membrane is possible. The photomicrographs clearly demonstrate that the sphere is constituted partly of oil and partly of the cell body. Of course, at this stage the diameter of the sphere reaches the maximum. The sphere obtained in the experiment strictly corresponds to that which has been presented in the theoretical scheme (Fig. 14 C).

The sucking force of the surface tension produced by the oil may, in fact, exceed the mechanical resistance of the cell membrane, and — as a result — the sphere breaks up exactly in the point opposed to the position of oil (Pl. XIII 55). That is just what has been predicted in the anticipated

scheme (Fig. 14 D). After breaking up the membrane, the cell disintegrates and simultaneously the diameter of the sphere clearly diminishes (Pl. XIII 56—58). Finally, only the oil drop of the initial diameter remains, with some residues of the amoeba's body (Pl. XIII 59).

The time and the final results of the phenomenon depend on the amount of oil injected. The experiment recorded photographically and described above lasted 3 hrs. 30 min.

The role of the quantity of oil is demonstrated by two photomicrographs taken both 5 min. after injection (Pl. XIV 60—61). As it could be deduced from the present theory, increasing of the amount of oil results in producing thicker and shorter forms of monopodial amoebae.

By means of a very abundant injection it is also possible to produce immediately the sphere (Pl. XIV 62). As it had been found in the case of injections of water, the sphere formed by amoeba and oil is also incapable of further blowing up and any excessive amount of oil destroys it.

The fact that the injection of a considerable quantity of oil transforms amoeba into a sphere, and that such a sphere bursts spontaneously after a certain time, or immediately after an excessive injection, provides further evidence for some conclusions drawn on the foregoing pages: 1. The oil drop is not "attached" to the cell membrane but it forms its constituent part. 2. The deficient amount of the surface material cannot be supplemented, during microinjection, by any rapid formation of a new membrane nor by any elastic stretch of the existing material.

On the contrary, all the results reported in this section seem to agree fully with the view that the cell surface of amoeba is a relatively permanent structure which can quickly adapt itself to the transformations of shape and size of the cell body only by the process of folding and unfolding.

Stereometrical effects

The last factor, the influence of which should be stressed in relation to the plasma-membrane ratio and the control of shape in amoeba, is purely geometrical in nature. If the cell body is flattened by a mechanical pressure, nothing would be changed in the plasma-membrane ratio. On the other hand however, at a constant plasma-membrane ratio, the flattened body which is forced to produce an extensive surface in one dimension, has no sufficient surface material available to develop any complicated form in two other dimensions. It might be expected therefore that, under such conditions, amoeba should assume initially a flat monopodial form, and subsequently — the discoidal shape.

As a matter of fact, the discoidal forms of amoebae had been produced in the cell compressor by Goldacre 1958. The present authors have also briefly reported such transformations of amoebae under simpler experimental conditions (Czarska and Grębecki 1965). In our procedure the cells were suspended in 1% methyl cellulose solution spread over the microscopic slide and covered with a cover-slip without any support. The cover-slip dips very slowly and presses gently the amoebae which ensures a gradual flattening of their body, lasting up to 30 min. In fact, fully in line with our views on the properties of the cell membrane in amoeba and with the concept of plasma-membrane ratio, at the first stage the flat monopodial

forms, and subsequently the discs, are produced. The shape transformations and the locomotory behaviour (rotary movement) manifested by amoebae under such conditions will be reported in full details elsewhere.

Another means of a mechanical control of the cell shape in amoeba is to dissect transversally a monopodial specimen. Such experiments have been performed by Seravin 1965 and 1966, and were repeated in the present research with the same results. The effect of bisection of a monopodial amoeba is shown by the Fig. 15. The anterior fragment very rarely retains the monopodial character but in most cases it assumes a spherical shape,

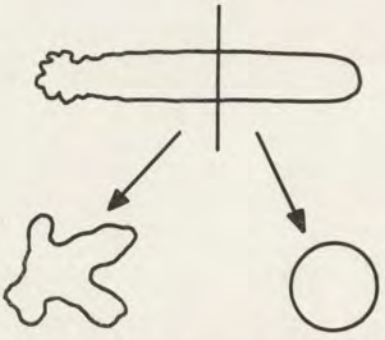

Fig. 15. Typical effect of bisection of a monopodial amoeba

while the posterior one — as a rule — produces a polypodial form. This result supports also the theory of membrane folding and plasma-membrane ratio: on the fore part of amoeba the membrane is unfolded, so that the anterior fragment is characterized by a very high plasma-membrane ratio and it should be transformed into a sphere; inversely, the membrane on the hinder part of amoeba is highly folded, and therefore the posterior fragment acquires a low plasma-membrane ratio which allows it to become polypodial.

Concluding discussion

Confrontation of the main theories

The theory of the stationary condition of the cell membrane in amoeba, and of its extremely rapid formation — resorption cycle, put forward by Goldacre 1961, seems to be accepted by the majority of recent authors (Bell, Chapman-Andresen, Jahn, Shaffer, Wohlfarth-Bottermann, should be cited in this respect). On the other hand many arguments of the classical theory of Jennings 1904 and Mast 1926, postulating that the membrane rolls around the body of the moving amoeba, are still valid and the concept of membrane rolling is advocated by other investigators (e.g. Abé, Allen, Seravin). The present theory is based on the view that the cell membrane in amoeba is a relatively permanent and non-extensible structure, which moves together with the whole cell by simultaneous folding and unfolding, and controls the cell shape according to the actual plasma-membrane ratio.

It seems useless to discuss here again all the arguments speaking in favour or against any of these theories, since they were exposed in details in the foregoing chapters. Instead, a summaric presentation of their compatibility with each theory is given in the Table 1.

It is the opinion of the present authors that the theory of membrane folding allows to reconciliate the controversive data which in part supported the first and in part the second of the former theories. It should be also pointed out that the theory of membrane folding and unfolding is closely connected with the views of Wolpert (Wolpert, Thompson and O'Neill 1964, and Wolpert 1965). Wolpert postulates also that the cell membrane is a rather permanent and non-extensible structure which moves with the whole cell; he believes that the membrane shift is due to some kind of flow. The essential concept is then the same, but the present theory tends to substitute a rather unprecise idea of membrane flow by the apparently more concrete mechanism of folding and unfolding. The theory of membrane folding and unfolding seems to have also some relation to the theory of movement of embryonic cells suggested by Holtfreter 1947 and that of locomotion of fibroblasts put forward by Ambrose 1961.

Nevertheless, some elements of membrane renewal as well as of membrane rolling may interfere with the essential mechanism of folding and unfolding. The part taken by this interference in the activity of amoeba will be shortly discussed in the following lines.

Membrane renewal and the uptake of food

It seems obvious that the assumption, supported in this article, that the membrane of amoeba is a permanent structure, has a limited validity, since the membrane material is in some extent gradually renewed during the cell life. Just this has been demonstrated by Wolpert and O'Neill 1962 in the experiment proving that the half-life period of the antibody labelled surface lasts about 5 hrs. The present authors oppose only the view that the membrane renewal would be so rapid that it might play any role in locomotion.

The most obvious mechanism of the membrane renewal is its turnover between the cell surface and cell inside, during different processes of phagocytotic or pinocytotic activity and — inversely — those of defecation of vacuolar structures (cf. the excellent review of Wohlfarth-Bottermann 1964). Some degree of the pinocytotic activity during locomotion has been stated in *Hyalodiscus simplex* by Wohlfarth-Botterman 1960 and in *Amoeba proteus* by Wolpert and O'Neill 1962. However, the observations of pinocytosis in moving amoebae reported in the present study seem to indicate that even in such a case this process is effected by the pinocytotic pseudopods and not by the tail itself, as it would be postulated by the theory of the stationary condition of the cell membrane. Much more information is available as to the phagotrophic activity of amoebae. It is well known that the food vacuoles are formed (i.e. a portion of the membrane is removed from the surface) in the anterior part of amoeba, and defecated (i.e. a portion of membrane is added to the surface) in the tail region. The formation of a vacuole at the front of organism and the defecation in its tail is — no doubt — the most suitable from the ecological point of view, and this is just opposite to the postulations of the theory of stationary condition of the cell mem-

Table 1

Consistency of various data with three main concepts of behaviour of the cell surface in amoeba. The facts considered are characterized as: unexplained by a theory or even contradictory to it — „−", explained or even postulated — „+", explained if some additionnal assumptions are made — „(+)'

Data considered	View on the permanence and mobility of the cell surface		
	Relatively permanent structure which rolls as a wheel caterpillar over the body (1)	Rapidly restorable structure which is stationary, being formed at the front and resorbed in the tail (2)	Relatively permanent structure which moves with the whole cell by folding and unfolding (3)
The tail appears coloured and pseudopods colourless in vital stains	−	+	+
The difference in colour arises immediately	−	−	+
The pseudopods reveal their colour after contraction	−	−	+
Half-life of the antibody-labelled surface lasts 5 hrs.	+	−	+
New pseudopods formed in a capillary are antibody-free	−	+	(+)
The particles laying at the substrate are not shifted by the amoeba moving over them	+	+	+
Amoebae overcrawling one upon another fail to affect mutually their movement	−	+	+
Glass spheres are at rest if they become a site of adhesion	+	+	+
Oil drop joining the membrane at the lateral side remains stationary	(+)	+	(+)
Glass fibres put upon the amoeba remain stationary	−	+	+
Glass spheres rotate if they touch the lateral side of amoeba	+	−	+
The pseudopod which touches the lateral side of another specimen turns in the direction of its movement	+	−	+
Glass spheres are pushed straight ahead by the tip of pseudopod	−	+	+
Oil drop joining the membrane at the tip of pseudopod is pushed straight ahead	−	+	+

Table 1 (continuation)

	(1)	(2)	(3)
Oil drop joining the membrane at the tail is not maintained on the surface, nor ingested, but squeezed off into the medium	−	−	+
Immobilization of the cell surface by a considerable increase of viscosity stops all the components of amoeboid movement	+	−	+
Small particles adhering to the cell surface move forwards	+	−	+
Big particles adhering to the cell surface accumulate on the tail	−	+	+
Particles laying at the substrate are never transferred upon the upper surface of amoeba	−	+	+
Small particles may be shifted forwards symmetrically from all sides	−	−	+
Symmetrical shift is rather apparent in relation to the cell but evident in relation to the ground	−	−	+
Sometimes an asymmetrical shift arises when some prticles are almost stationary in relation to the ground but the others move faster than the cell does	+	−	+
The distance between two particles laying on the axis of the same pseudopod may oscillate	−	−	+
The distance between two particles laying on two neighbour pseudopods increases	−	(+)	+
A moving amoeba promotes conspicuous streamings in the viscous medium	+	−	+
The effect of streamings is "amplificated" by the increase of viscosity	+	−	+
The streamings near the lateral body side correspond to the direction of movement	+	−	+
General pattern of streamings is essentially a system of whirles, symmetrical in respect to the axis of movement of amoeba	−	−	+
In the symmetrical system of whirles the velocity of the extracellular streaming never exceeds the rate of cell locomotion	−	−	+
Asymmetrical system of whirles may sometimes arise in relation with the asymmetry of movement	+	−	+

Table 1 (continuation)

	(1)	(2)	(3)
In the asymmetrical system of whirles the velocity of the extracellular streaming exceeds the rate of cell locomotion	+	−	+
Decrease in the cytoplasm volume induced osmotically or by microinjection is not compensated by a resorption of surface but the amoeba becomes more polypodial	+	−	+
Increase in the cytoplasm volume induced osmotically or by microinjection is not compensated by an addition of surface but the amoeba becomes less polypodial, monopodial, or spherical	+	−	+
Decrease in the amount of surface material by pinocytosis or by surface tension of an oil drop is not compensated but the amoeba becomes monopodial or spherical	+	−	+
Spherical amoeba is incapable to supplement its surface material and it is incapable to further blowing up	+	−	+
A flattened amoeba is incapable to supplement the deficient surface material and it preserves the discoidal form	+	−	+
Fragments of a monopodial amoeba after bisection cannot rapidly adjust the amount of the surface material but become spherical and polypodial respectively	+	−	+

brane. At any rate, all these precesses of the pinocytotic or phagocytotic activity, in moving amoebae, seem to be too slow to be considered a factor of locomotion. On the contrary, when they intensify, e.g. when the pinocytosis is experimentally induced — all the locomotory activity ceases.

Membrane rolling and the substitution of pseudopods

As it has already been pointed out above, some elements of the rolling phenomena may accompany folding and unfolding of the cell surface of amoeba. As well shifting of the small granules adhering to the cell surface as the streamings arising by pulling the viscous medium, are not alwyas symmetrical: it happens sometimes that the movement ceases at one side of amoeba and at the other side it reaches a speed exceeding the rate of the cell locomotion.

Facultative arising of some temporary rolling effects is not contradictory but is even postulated by the theory of membrane folding and unfolding. The subject is closely related to the problem of a continuous substitution of existing pseudopods by the new ones. As it has been postulated above (cf. Fig. 3), the pseudopod stops its further extension when there is no more folded material available to unfold and cover its growing area. Then a new pseudopod arises in another site of the cell surface.

It seems rather obvious that, if the new pseudopod protrudes from the region rich in the folded surface material at all sides, the membrane may unfold rather symmetrically, as it is suggested in fact by the majority of our

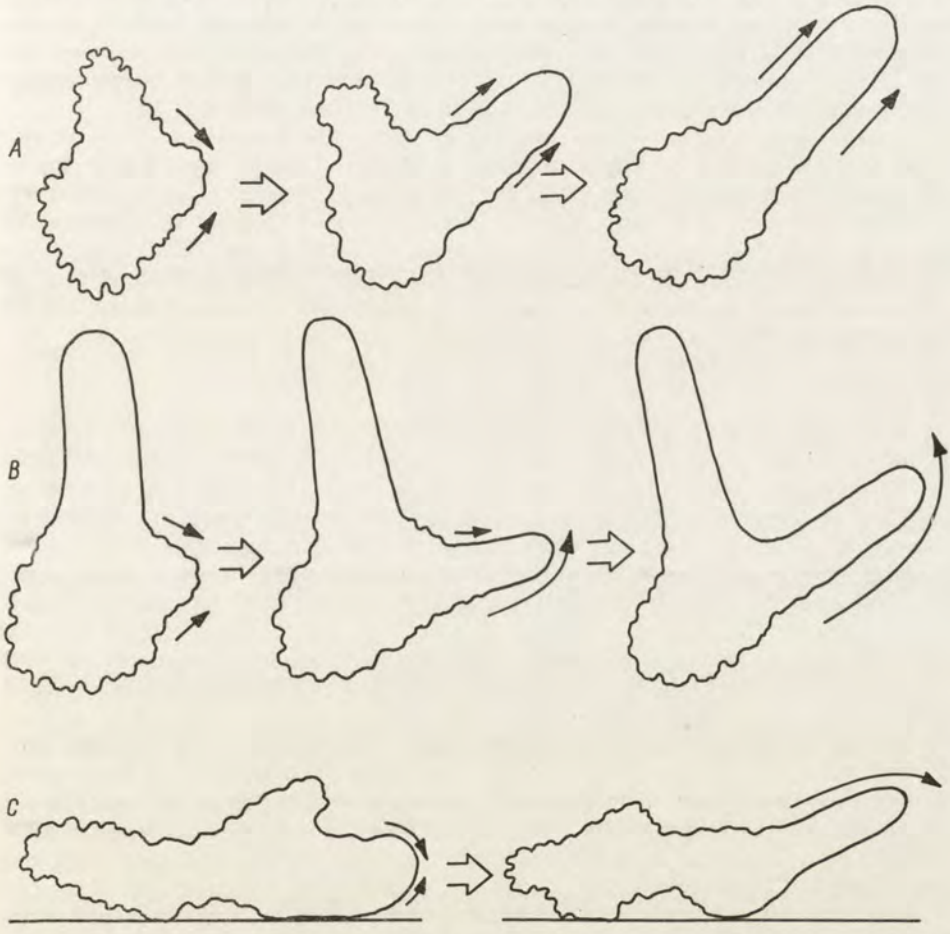

Fig. 16. Diagram explaining the possible variants of membrane unfolding during the extension of a new pseudopod. A. Quite symmetrical unfolding occurring when the sufficient amount of the folded material is present all around the new pseudopod. B. A horizontal rolling effect resulting from the asymmetrical unfolding, in the case when the membrane is highly folded at the one side of new pseudopod and rather stretched at its other side. C. A vertical rolling effect occurring when the new pseudopod is formed near the site of adhesion to the substrate which impedes the unfolding from that side

experiments with adhering granules and viscous media. Such a behaviour of the cell surface is diagrammatically represented by the Fig. 16 A.

Situation is quite different when the new pseudopod arises in such an area which is rich in the folded surface material at one side but very poor in it at the other, e.g. in the vicinity of the former leading pseudopod with its surface still unfolded. As this is indicated by the Fig. 16 B, under such conditions the membrane cannot unfold from one side, and it should unfold at higher rate from the other side instead. As a result, an effect of rolling should occur, which could be called a horizontal rolling to distinguish it from the vertical rolling postulated by the classical theories.

The possibility of arising of a vertical rolling could also be predicted by the theory of membrane folding and unfolding. It should arise if a new pseudopod is formed near the point adhering to the substrate, because the unfolding is impossible in the site of adhesion and this should be compensated by a more intense unfolding of the upper surface (see Fig. 16 C).

It has been stressed before by one of the present authors (Grębecki 1964) that the theory of the permanent vertical rolling is inapplicable to the polypodial amoebae, because of purely geometrical reasons. On the contrary, it seems evident that if one considers the rolling as a facultative phenomenon which is secondary to the essential process of folding and unfolding of the cell surface, it helps to understand the behaviour of the cell membrane in polypodial amoebae and the mechanism of substitution of one pseudopods by the others.

Summary

Re-examination of the vital staining experiments puts in question their validity to prove the stationary condition of the cell membrane in amoeba. The big obstacles are indeed motionless but the delicate ones are shifted by the cell surface. Big particles adhering to the surface accumulate in the tail but the small ones move forwards. This movement is in most cases symmetrical and then its speed fails to exceed the rate of locomotion, which speaks against the theory of membrane rolling. The rolling effects may be manifested as a secondary phenomenon related to an asymmetry of cell movement and then the resting state of particles at one side is compensated by doubling their speed at the other side.

The shifting cell membrane pushes and pulls the viscous medium producing big whirles around the body. The pattern of whirles is also essentially symmetrical and then the extracellular streaming is never more rapid than the cell movement, and also an asymmetry may occasionally arise reflecting the changes of the character of cell movement and producing the secondary rolling effects.

The surface area in amoeba cannot be supplemented by any rapid addition of new membrane material nor by an elastic stretch. As a result, every change in the proportion between the cytoplasm volume and the membrane area (plasma-membrane ratio) may be compensated only by shape transformation. The plasma-membrane ratio has been experimentally decreased by a decrease of the cytoplasm volume (incubation in hypertonic media). It has been also increased, as well by an increase of the cytoplasm volume (incubation in hypotonic media and microinjections), as by a decrease of the mem-

brane area (its ingestion by pinocytosis and "sucking" by an oil drop). Every decrease in the plasma-membrane ratio mades the amoeba more polypodial, whereas the increase of this ratio transforms its shape in the direction: less polypodial → monopodial → spherical.

It is concluded that the cell membrane in amoeba is not a rapidly restorable structure which could be constantly made at the front, resorbed in the tail, and remain stationary at the sides. Also its rolling over the body is not a constant and essential phenomenon but a rather occasionnally and secondary effect. The cell membrane is a relatively permanent structure which may move forwards with the whole cell with the total average velocity equal to the rate of locomotion. It shifts forwards by the simultaneous expansion in some areas and collapse in anothers. The cell surface in amoeba is distinctly convoluted, and therefore its expansion may be explained by unfolding and the collapse — by re-folding.

STRESZCZENIE

Kontrola doświadczeń opartych na barwieniu przyżyciowym stawia w wątpliwość ich wartość dowodową dla teorii stacjonarności błony komórkowej u ameb. Duże przeszkody istotnie pozostają w bezruchu, ale subtelne są przesuwane przez błonę komórkową. Duże cząstki przywierające do powierzchni zbierają się w tylnej części ameby, ale drobne wędrują do przodu. Wędrówka ich jest przeważnie symetryczna, a wówczas jej szybkość nie przekracza szybkości lokomocji, co przemawia przeciwko teorii gąsienicowego przetaczania się błony. Efekty przetaczania mogą ujawniać się jako zjawisko wtórne związane z asymetrią ruchu komórki; właśnie wówczas spoczynkowy stan cząstek po jednej stronie jest kompensowany przez podwojenie ich szybkości z drugiej strony.

Przesuwająca się błona komórkowa pociąga za sobą lepkie środowisko powodując powstawanie rozległych wirów wokół ciała. Układ wirów jest również zasadniczo symetryczny i wówczas prąd w środowisku nigdy nie wyprzedza ruchu komórki, ale i tym razem może powstawać chwilowa asymetria odzwierciedlająca zmiany charakteru ruchu i wywołująca wtórne efekty przetaczania błony.

Powierzchnia komórki u ameby nie może być uzupełniana ani przez szybkie dodawanie nowych elementów błony ani przez elastyczne rozciąganie. Wskutek tego każda zmiana proporcji między objętością cytoplazmy a powierzchnią błony (stosunek plazmo-membranowy) może być skompensowana tylko zmianą kształtu. Stosunek plazmo-membranowy był doświadczalnie obniżany przez zmniejszanie objętości cytoplazmy (inkubacja w środowiskach hypertonicznych). Był on też podwyższany zarówno przez zwiększanie objętości cytoplazmy (inkubacja w środowiskach hypotonicznych oraz mikroinjekcje), jak i przez zmniejszanie powierzchni błony (wchłanianie jej w toku pinocytozy oraz „wsysanie' przez mikrokroplę oleju parafinowego). Każde obniżenie stosunku plazmo-membranowego nadaje amebie większą polypodialność, podczas gdy podwyższenie tego stosunku zmienia jej kształt w kierunku: mniejsza polypodialność → monopodialność → sferyczność.

Autorzy dochodzą do wniosku, że błona komórkowa ameby nie jest strukturą natychmiast odtwarzalną, która mogłaby być wciąż wytwarzana u przodu komórki, resorbowana w tyle, a pozostawać nieruchoma po bokach. Również jej

przetaczanie się wokół komórki nie jest zjawiskiem stałym i podstawowym, lecz raczej chwilowym i wtórnym. Błona komórkowa jest względnie trwałą strukturą, która może posuwać się naprzód wraz z całą komórką zachowując średnią globalną szybkość równą szybkości jej lokomocji. Posuwa się ona do przodu dzięki jednoczesnemu rozciąganiu w jednych okolicach a ściąganiu w innych. Powierzchnia komórki ameby jest silnie pomarszczona, wobec czego jej rozciąganie tłumaczy się jako rozfałdowywanie, a ściąganie — jako ponowne fałdowanie.

REFERENCES

Abé T. 1962: Morpho-physiological study of ameboid movement. II. Ameboid movement and the organization pattern in a striata ameba. Cytologia, Tokyo 27, 111—139.

Ambrose E. J. 1961: The movement of fibrocytes. Exp. Cell Res. (suppl.) 8. 188—198.

Bell L. G. 1961: Surface extension as the mechanism of cellular movement and cell division. J. theoret. Biol. 1, 104—106.

Brandt P. W. and Pappas G. D. 1960: An electron microscopic study of pinocytosis in ameba. I. The surface attachment phase. J. biophys. biochem. Cytol. 8, 675—687.

Chapman-Andresen C. 1962: Studies on pinocytosis in amoebae. C. R. Lab. Carlsberg 33, 73—264.

Chapman-Andresen C. 1964: Surface renewal in *Amoeba proteus*. J. Protozool. (suppl.) 11, Abstr. 14.

Czarska L. and Grębecki A. 1965: Rotary movement in *Amoeba proteus*. Progress in Protozoology, Abstr. Second int. Conf. Protozool., London 1965, Excerpta med. Int. Congr. ser. No 91, Abstr. 327.

Goldacre R. J. 1952: The folding and unfolding of protein molecules as a basis of osmotic work. Int. Rev. Cytol. 1, 135—164.

Goldacre R. J. 1958: The regulation of movement and polar organisation in *Amoeba* by intracellular feedback. In: Proc. Ist int. Congr. Cybernetics, Namur 1956. Gautier-Villar, Paris 1958.

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. Exp. 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, 497—499.

Grębecki A. 1961: L'enregistrement microphotographique des courants d'eau autor d'un Cilié. Experientia 17, 93—94.

Grębecki A. 1964: Modern lines in the study of amoeboid movement. Acta Protozool. 2, 379—402.

Griffin J. L. and Allen R. D. 1959: Movements of the cell membrane of some fresh- and salt-water amebae. Biol. Bull. 117, 394—395.

Holtfreter J. 1947: Observation on the migration, aggregation and phagocytosis of embryonic cells. J. Morph. 80, 25—55.

Jahn T. L. 1964: Relative motion in *Amoeba proteus*. In: Primitive motile systems in cell biology. Academic Press, 279—302.

Jennings H. S. 1904: Contributions to the study of the behavior of lower organisms. VI. The movements and reactions of *Amoeba*. Carnegie Inst. Wash. Pub. 16, 129—234.

Jeon K. W. and Bell L. G. 1964: Behaviour of cell membrane in relation to locomotion in *Amoeba proteus*. Exp. Cell Res. 33, 531—539.

Kanno F. 1964: An analysis of amoeboid movement. II. Mechanical properties of surface structure of *Amoeba*. Annot. zool. jap. 37, 12—19.

Kanno F. 1965: An analysis of amoeboid movement. IV. Cinematographic analysis of movement of granules with special reference to the theory of amoeboid movement. Annot. zool. jap. 38, 45—63.

Landau J. V. 1965: High hydrostatic pressure effects on *Amoeba proteus*. J. cell Biol. 24, 332—336.
Mast S. O. 1926: Structure, movement, locomotion and stimulation in *Amoeba*. J. Morph. 41, 347—425.
McClendon J. F. 1909: Protozoan studies. J. exp. Zool. 6, 265—283.
O'Neill C. H. 1964: Isolation and properties of the cell surface membrane of *Amoeba proteus*. Exp. Cell Res. 35, 477—496.
Pappas G. D. 1954: Structural and cytochemical studies of the cytoplasm in the family *Amoebidae*. Ohio J. Sci. 54, 195—222.
Ponder E. 1948: Haemolysis and related phenomena. Grune and Stratton, New York.
Prescott D. M. 1953: Relation between dye uptake and cytoplasmic streaming in *Amoeba proteus*. Nature 172, 593.
Rinaldi R. A. and Jahn T. L. 1963: On the mechanism of ameboid movement. J. Protozool. 10, 344—357.
Schaeffer A. A. 1920: Amoeboid movement. Princeton Univ. Press, Princeton, New Jersey.
Schneider L. und Wohlfarth-Bottermann K. E. 1959: Protistenstudien IX. Elektronenmikroskopische Untersuchungen an Amöben unter besonderer Berücksichtigung der Feinstruktur des Cytoplasmas. Protoplasma 51, 377—389.
Seravin L. N. 1964: Kritičeskij obzor sovremennych predstavlenij ob ameboidnom dviženii. Citologija 6, 653—666.
Seravin L. N. 1965: A hypothesis of the multiple mechanisms providing for the ameboid movement. Progress in Protozoology, Abstr. Second int. Conf. Protozool., London 1965, Excerpta med. Int. Congr. ser. No 91, Abstr. 109.
Seravin L. N. 1966: Monopodialnye formy *Amoeba proteus*. Dokl. Akad. Nauk SSSR 166, 1472—1475.
Shaffer B. M. 1963: Behaviour of particles adhering to amoebae of the slime mould *Polysphondylium violaceum* and the fate of the cell surface during locomotion. Exp. Cell Res. 32, 603—605.
Shaffer B. M. 1965 a: Antistrophic pseudopodia of the collective amoeba *Polysphondylium violaceum*. Exp. Cell Res. 37, 79—92.
Shaffer B. M. 1965 b: Cell movement within aggregates of the slime mould *Dictyostelium discoideum* revealed by surface markers. J. Embryol. exp. Morphol. 13, 97—117.
Wohlfarth-Bottermann K. E. 1960: Protistenstudien X. Licht-und elektronenmikroskopische Untersuchungen an der Amöbe *Hyalodiscus simplex* n. sp. Protoplasma 52, 58—107.
Wohlfarth-Bottermann K. E. 1964: Cell structures and their significance for ameboid movement. Int. Rev. Cytol. 16, 61—131.
Wolpert L. 1965: Cytoplasmic streaming and amoeboid movement. Symp. Soc. gen. Microbiol. 15, 270—293.
Wolpert L. and O'Neill C. H. 1962: Dynamics of the membrane of *Amoeba proteus* studied with labelled specific antibody. Nature 196, 1261—1266.
Wolpert L., Thompson C. M. and O'Neill C. H. 1964: Studies on the isolated membrane and cytoplasm of *Amoeba proteus* in relation to ameboid movement. In: Primitive motile systems in cell biology. Academic Press, 143—168.

Note added in proof

A few months after submitting this paper to the Editors, appeared the important study of Wohlfarth-Bottermann K. E. und Stockem W. 1966: Pinocytose und Bewegung von Amöben. II. Permanente und induzierte Pinocytose bei *Amoeba proteus*. Zeitschr. Zellforsch. 73, 444—474.

The findings of the above authors seem to corroborate our views and results in many respects: 1. The behaviour of Latex spheres on the cell surface supports our explanation of experiments with other granular surface markers. 2. Blocking of amoeboid movement by the substances adsorbing on the mucous layer seems to be similar to its blocking in viscous medium. 3. The form of pinocytosis intermediary between the induced and the permanent one, evoked by alcian blue,

strictly corresponds to the picture presented by us for the same inductor. 4. The cyclic appearance of the permanent pinocytosis is probably related to the cycle of membrane re-folding in the tail, as postulated by our theory. 5. The calculated rate of membrane ingestion in the tail (1—10% per 30 min.) is absolutely insufficient to make the cell surface stationary, which strongly supports our opinion that the membrane resorption-restoration cycle plays a rather secondary role in locomotion.

EXPLANATION OF PLATES I—XIV

Streamings surrounding the amoebae in viscous medium

1 : Two amoebae simultaneously seen in the field, each surrounded by its proper system of streamings. Medium at rest on the margins of the field

2—3: General picture of streamings clearly demonstrating the pattern of whirles. Arrows indicate the extending and contracting pseudopods

4—5: Two successive registrations of streamings around the same specimen demonstrating the facultative temporary asymmetry of whirles; *a* — initial leading pseudopod, *b* — pseudopod which has taken the initiative during the second registration

6: Symmetrical character of streamings around a pseudopod during its normal uniform extension

7: Asymmetry of the streaming surrounding a pseudopod which was changing the direction of its extension

8: A rather symmetrical pattern of streamings accompanying the tail region during its uniform contraction

9: Distinctly asymmetrical pattern of streamings near the tail region which manifested a lateral contraction

10: High magnification of the paths followed by a group of suspended crystals to demonstrate the presumable rhytmicity of pulling the medium by amoeba

11—12: Streamings promoted in the medium by monopodial forms of amoebae

13: Resting state of particles laying at the substrate beneath the amoeba (normal medium without any increase of viscosity)

Common shapes of *Amoeba proteus*

14: A typical polypodial specimen. Phase contrasting optics. The dark field effect has been added to this of the phase contrast by applying a reddish illumination and taking picture on the plate insensitive to the red

15: A typical monopodial specimen taken from normal culture. The same technique as in 14

Shape transformations under the osmotic effects

16: Shape of amoebae one day after the transfer from the normal culture to the Chalkley medium concentrated 2×. Note the polypodial character

17: Amoebae transferred from the Chalkley medium concentrated 2× to the distilled water. Note the attenuation of the polypodial pattern

18: Shape of amoebae one day after the transfer from the Chalkley medium concentrated 2× to that concentrated 4×. Note the distinct increase of the polypodial character

19: Amoebae transferred from the Chalkley medium concentrated 4× to the distilled water. Note the approach to the monopodial form

20: Shape of amcebae one day after the transfer from the Chalkley medium concentrated 4× to that concentrated 8×. Note the rosette shape

21: Amoebae transferred from the Chalkley medium concentrated 8× to the distilled water. Note their nearly monopodial form

22: Shape of amoebae one day after the transfer from the Chalkley medium concentrated 8× to that concentrated 16×. Note the rosette forms (22 a) and the "ghosts" of disintegrated cells (22 b)

23: Amoebae transferred from the Chalkley medium concentrated 16× to the distilled water. Note the occurrence of smooth spheres

Microinjection of 100 mM NaCl

24: The micropipette inserted into a polypodial amoeba

25: Amoeba starts to move during the injection

26: Amoeba shifts away from the pipette
27: Amoeba in the first minuts after injection, still preserving its polypodial shape
28: Amoeba 5 min. later, after imbibing water, increases in size and becomes nearly monopodial
29: 30 sec. later, when probably the ion regulation took place, amoeba recovers its former size and the polypodial shape

Microinjection of the culture medium

30: Micropipette inserted into a polypodial specimen
31: Amoeba blows up as a baloon during a massive injection
32: Injection of further amount of the Chalkley medium. Amoeba becomes perfectly monopodial, lacking only the hyaline cap
33: Another specimen, after so massive injection of the Chalkley medium as to produce the spherical form
34—35: Bursting of the spherical form after injection of an excessive amount of the Chalkley medium

Shape transformations after pinocytosis in alcian blue

36: Polypodial specimen from the original culture
37: Monopodial specimen from the original culture
38: Nearly monopodial specimens arising after the pinocytosis in 1 : 10 000 solution of alcian blue
39: Perfectly monopodial specimen, lacking only the hyaline cap, produced under the same conditions (the most common form)
40: Perfectly monopodial specimen, bearing the typical hyaline cap, produced under the same conditions (note the distinct difference in size in respect to the monopodial form from the the original culture)
41: Spherical form produced under the same conditions

Microinjection of oil

42: Micropipette inserted into a polypodial specimen
43: Moment of injection. Note the "channel" linking the oil drop with the tip of the pipette. Oil forms the leading cap of amoeba already during the injection
44: Stretching of the cell membrane near the oil drop soon after the injection
45—47: Three successive stages of the next period when amoeba assumes a perfectly monopodial form
48—49: Gradual shortening of the monopodial amoeba indicating that it begins to form with the oil a joint sphere. Note that the diameter of the sphere starts to increase
50—51: Further increase of the sphere. Only the tail of amoeba remains still outside
52—54: Three successive stages when the amoeba with oil becomes nearly spherical and finally forms an ideal sphere. The diameter of the sphere reaches the maximum. Constitution of the sphere partly by oil and partly by the cell body is clearly seen
55: Breaking of the cell membrane at pole exactly opposite to the position of oil
56—58: Three stages of the disintegration of the cell. Note the gradual diminution of the diameter of the sphere
59: Remaining oil drop of the initial size
60—61: Two other specimens with injected different amount of the oil to demonstrate the difference in shape of the monopodial stage
62: Sphere composed partly of oil and partly of the cell body, produced immediately by massive injection

Some aspects of membrane folding

63: Difference in the degree of membrane folding in the posterior and anterior part of amoeba
64: Difference in the velocity of movement of front and the rear end of a monopodial specimen. Compare the long paths described by the granules in the tail with the small distance covered in the same time by the hyaline cap (thickness of the bright crescent at the right of the picture)

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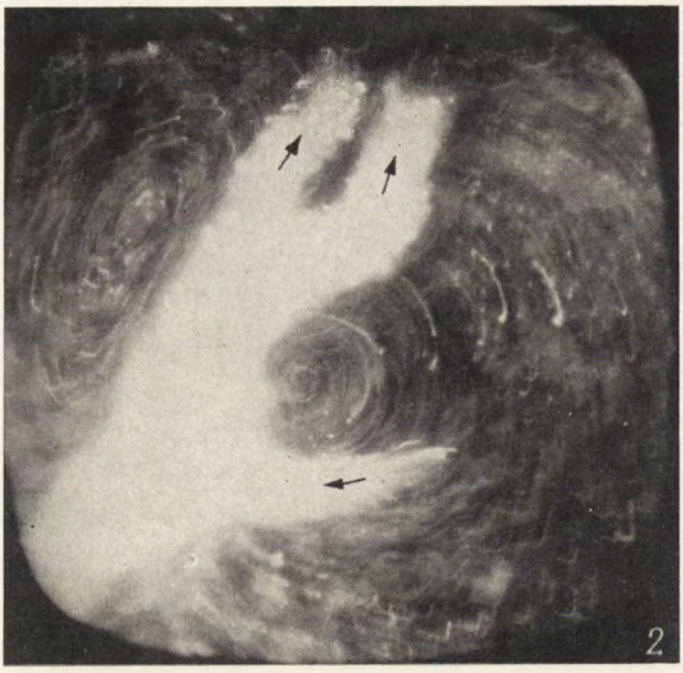

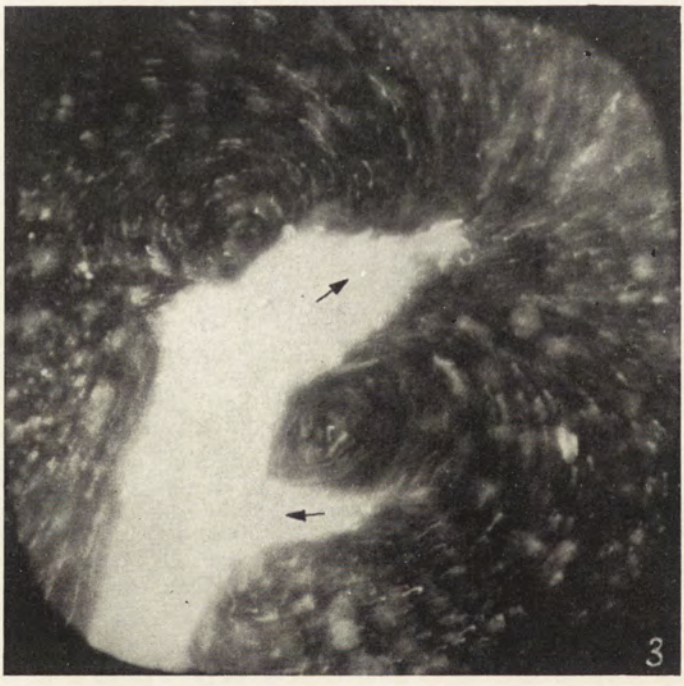

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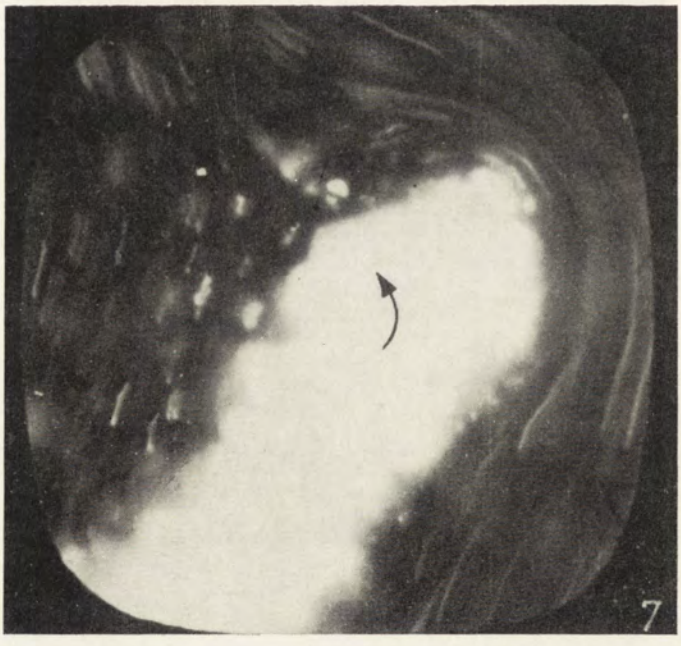

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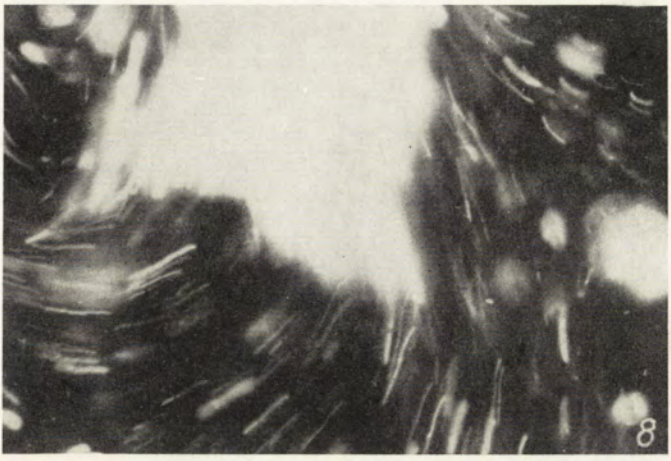

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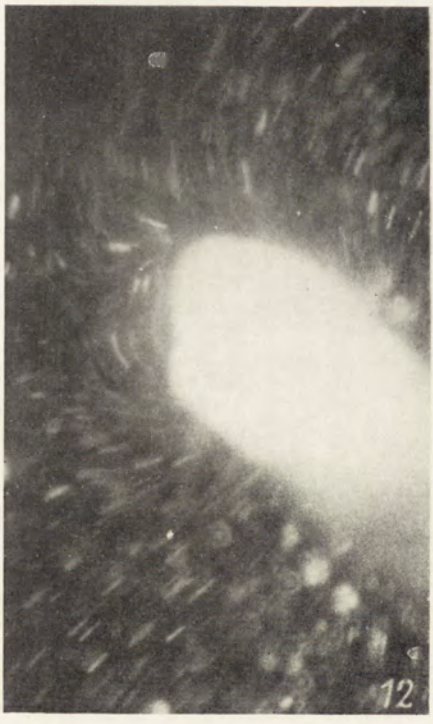

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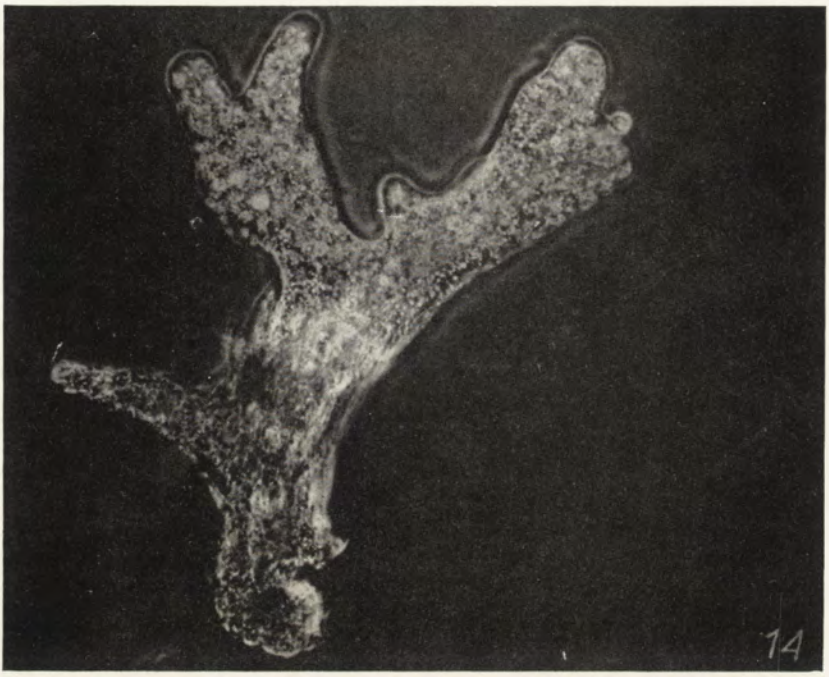

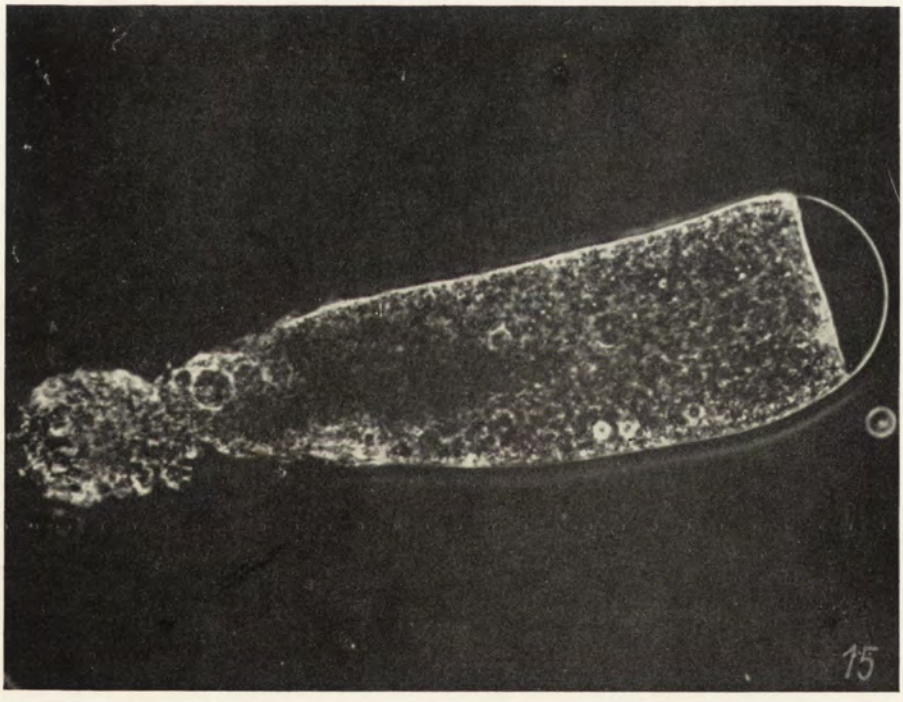

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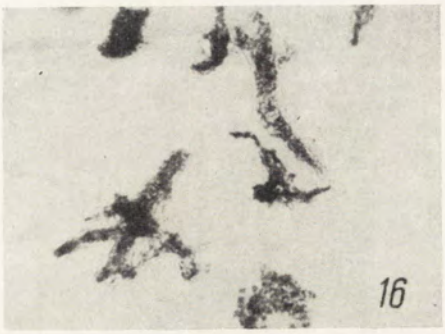

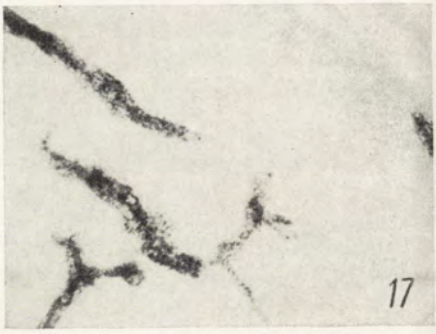

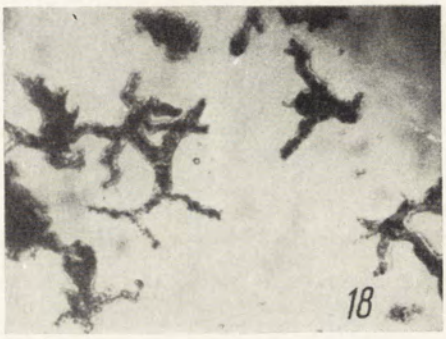

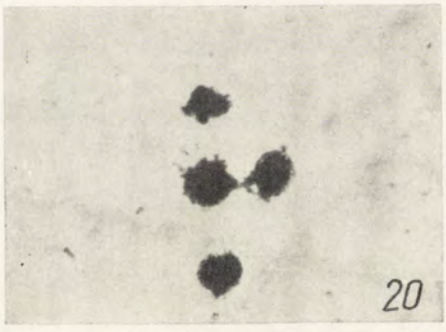

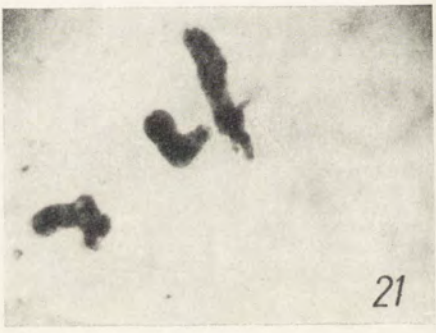

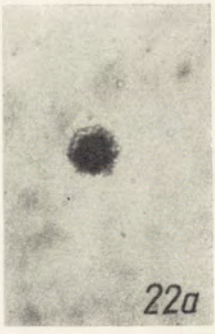

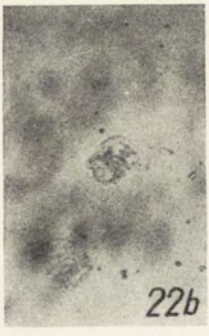

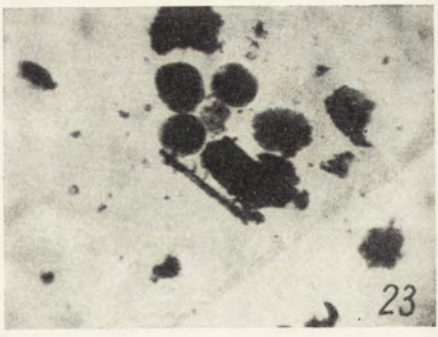

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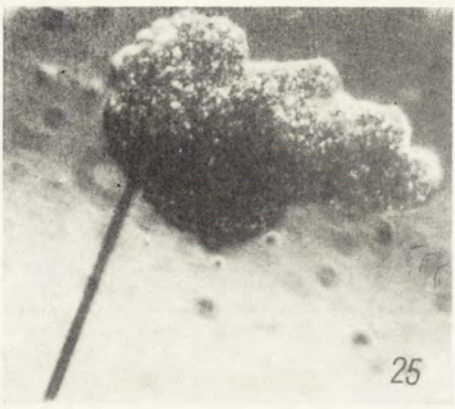

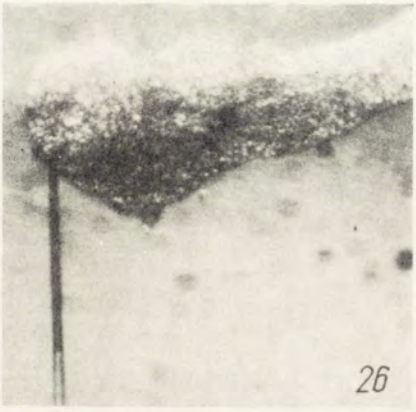

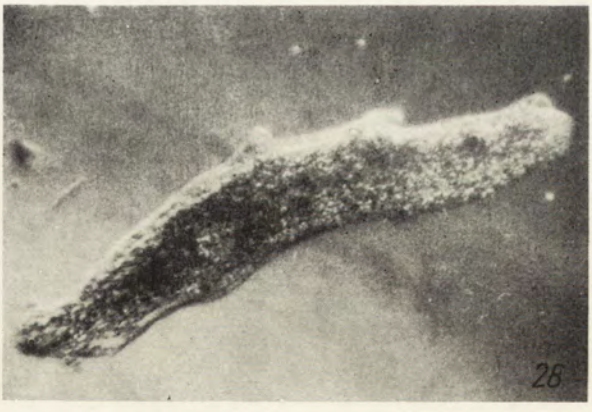

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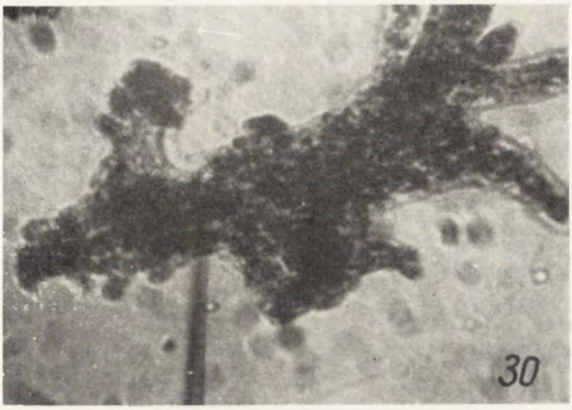

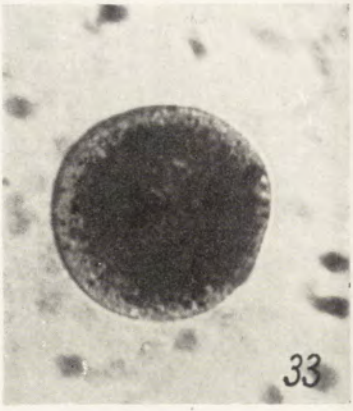

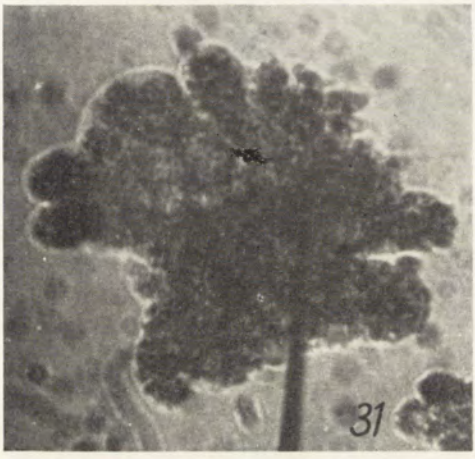

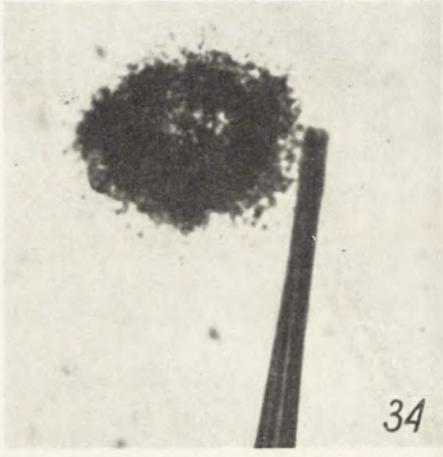

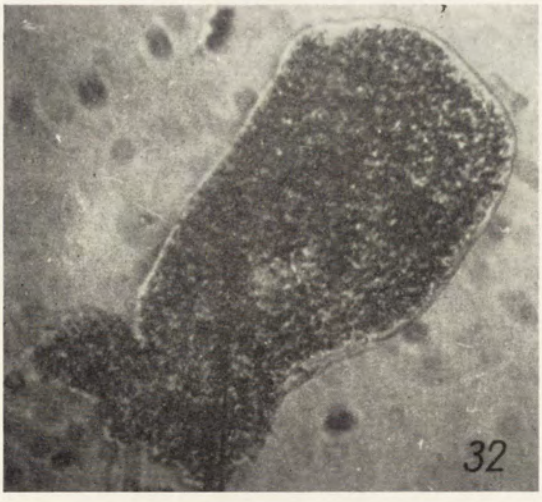

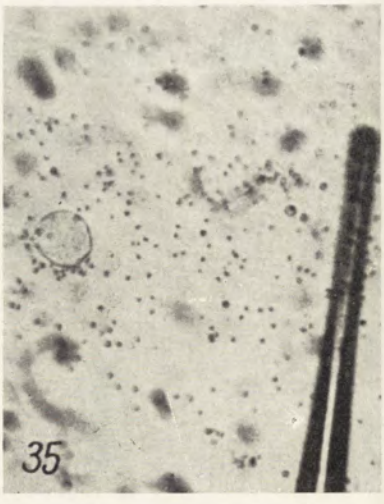

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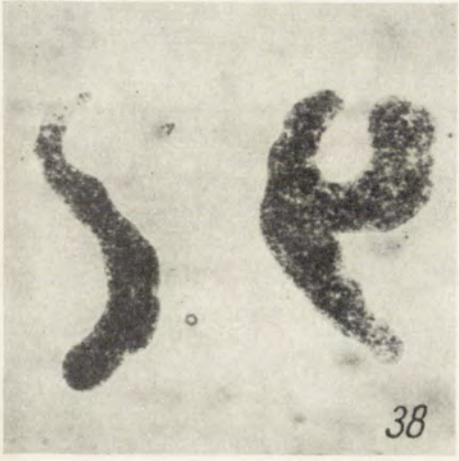

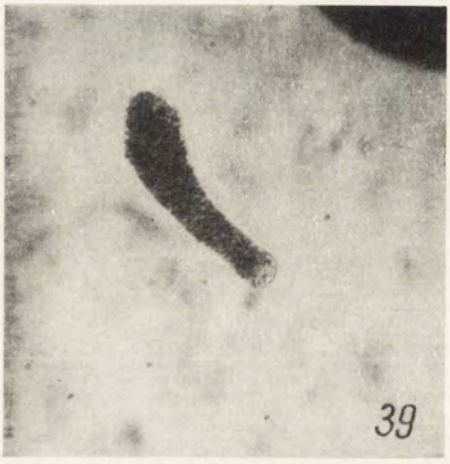

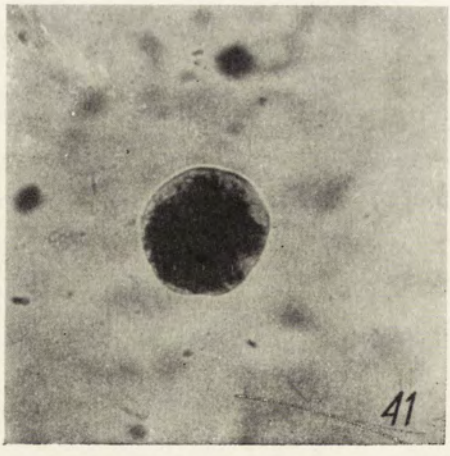

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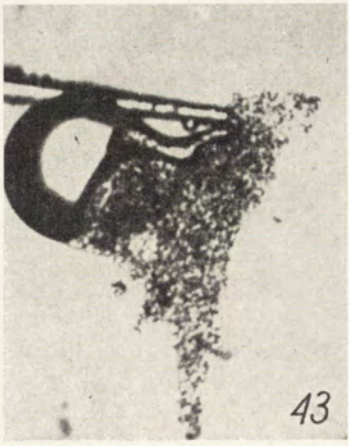

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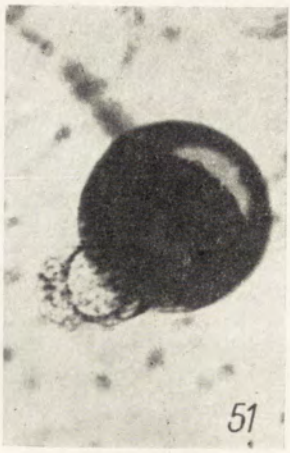
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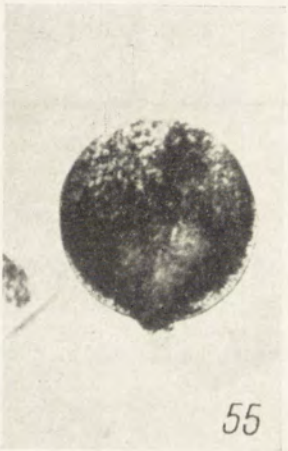
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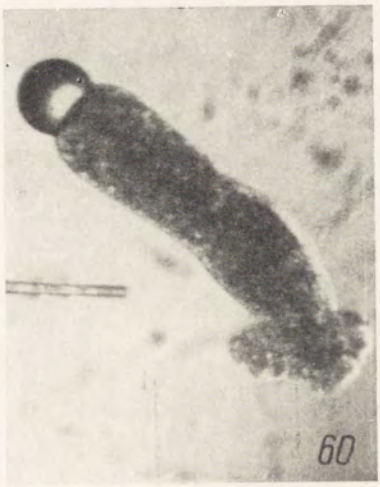

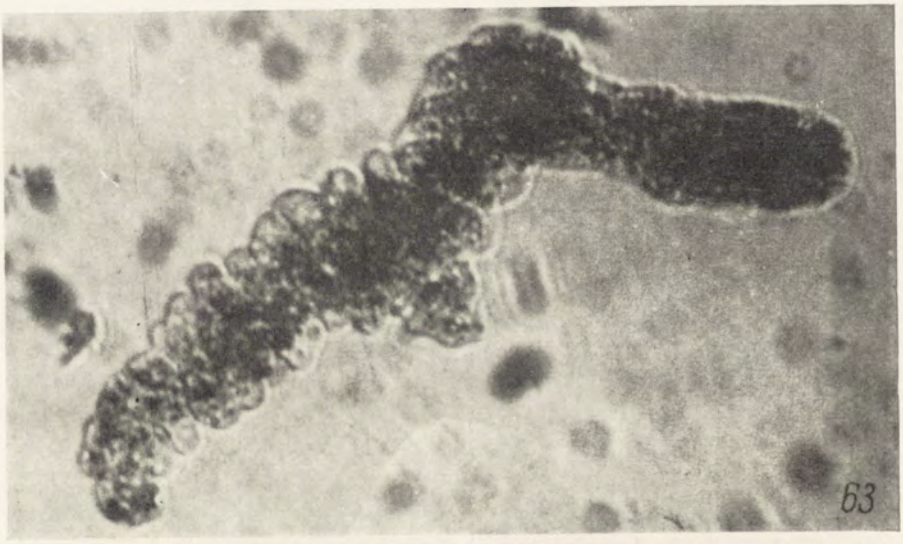

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Leszek KUŹNICKI

Role of Ca^{2+} ions in the excitability of protozoan cell. Calcium factor in the ciliary reversal induced by inorganic cations in *Paramecium caudatum*

Znaczenie jonów Ca^{2+} w pobudliwości komórki pierwotniaczej. Rola wapnia w rewersji rzęskowej wywołanej u *Paramecium caudatum* przez kationy nieorganiczne

The important role of calcium in the ciliary reversal induced by potassium in *Paramecium* was first experimentally demonstrated by Kamada 1938, 1940 and by Kamada and Kinosita 1940. According to Kamada 1940, beckward swimming occurs when the calcium content inside the cell has decreased. The calcium loss theory was developed and modernized by Jahn 1962. He explained the ciliary reversal induced by some inorganic cations, by oxalate, and cathodal current, as result of removal of Ca^{2+} from the cell membrane of paramecia.

Just recently the calcium content hypothesis of the ciliary reversal and of other motoric responses of *P. caudatum* have been put forward by Grębecki 1965. As it was experimentally demonstrated by this author (Grębecki 1965), a gradual decalcification of ciliates by application of EDTA, citrates, oxalates in the medium involves the following sequence of motoric responses: normal movement (NM), periodic ciliary reversal (PCR), continuous ciliary reversal (CCR), partial ciliary reversal (PaCR), normal movement (NM).

When after a previous treatment with EDTA, paramecia are recalcified by a gradual increase of the calcium content in the medium, all the typical responses occur in an inverse sequance: NM → PaCR → CCR → PCR → NM. Considering these facts as well as some others, Grębecki 1965 postulated that ciliary reversal is released not directly by the loss of calcium but by a certain degree of calcification which is established in the ciliate membrane (calcium content hypothesis). Above and below this range cilia work normally.

In the same laboratory and simultaneously with the study of Grębecki on the Ca-chelating and Ca-precipitating agents, the motor responses of *P. caudatum* immediately after addition of thalium salts to the medium were under study. Specimens in calcium solutions were treated with calcium-thalium mixtures (the level of calcium was the same as in the initial medium). The gradual increase of the Tl_2SO_4 concentration evoked in paramecia a series of

ciliary responses as follows: NM → PCR → CCR → PaCR → NM, which is the same sequence as that after application of EDTA, citrate or oxalate. This result strongly suggests that all the motor responses induced in *Paramecium* by various cations may follow essentially the same mechanism as that produced by Ca-chalting and Ca-precipitating agents. The aim of this research was to prove experimentally this hypothesis.

Methods

Experiments were carried out on a clone of *P. caudatum* grown in milk containing medium. Ciliates taken from a thigmotactic ring were rinsed four times in the definitive $CaCl_2$ solutions until the initial medium became diluted 10.000 times. Three standard solutions of Ca were applied: 5.0 mM, 0.5 mM, 0.05 mM. Paramecia were used for experiments not sooner than 24 hrs. after rinsing in calcium solutions.

The motor responses of ciliates were investigated in solutions of following inorganic salts: LiCl, NaCl, KCl, RbBr, CsCl, Tl_2SO_4, $MgCl_2$, $CaCl_2$, $MnCl_2$, $SrCl_2$, $BaCl_2$. 0.5 ml of standard calcium solutions containing about 100 individuals was mixed with 0.5 ml of a defined solution of the studied substance prepared at the same calcium level. The whole experimental procedure was performed at 20 ± 1°C:

The ciliary response which appears in the first minute after the factor under study has been added and lasts no less than 30 sec., was accepted in the first place as base of evaluation. It was stated that under the conditions studied motor reactions of ciliates appear in which four different types may be distinguished: normal movement (NM), periodic ciliary reversal (PCR), continuous ciliary reversal (CCR), partial ciliary reversal (PaCR).

All that types of ciliary response in *Paramecium* have been described by many authors and a full comparative revue of their results has been recently reported by Grębecki (1965). In this article the discussion of this problem will be reduced to its general characteristics.

1. Normal movement: the ciliate swims forwards with its anterior body part spiralizing left. The path described by the ciliate has the form of an elongate spiral.

2. Periodic ciliary reversal: the ciliate changes periodically its manner of swimming, most frequently at intervals of 0.5—2 sec. From the forward movement it passes to short-lasting ciliary reversal which terminates as a turn at spot followed by forward swimming again, and by a subsequent reversion, etc. The path covered by the ciliate in the periodic ciliary reversal resembles most frequently a "zig-zag" composed of crossing lines.

3. Continuous ciliary reversal: ciliate swims forwards with its posterior body end, spiralizing left.

The path described by the ciliate has a shape of a strongly wound spiral.

4. Partial ciliary reversal: the ciliate describes circles inclining to the dorsal side. The shape of its path approximates a circle.

The types of movement described above are illustrated in Fig. 1. For establishing precisely the motor reactions of *Paramecium* in the range of concentrations at which one motor type passed to another, the Dryl's method of photomacrographic registration of ciliates movement was applied besides the observation in stereoscopic microscpe.

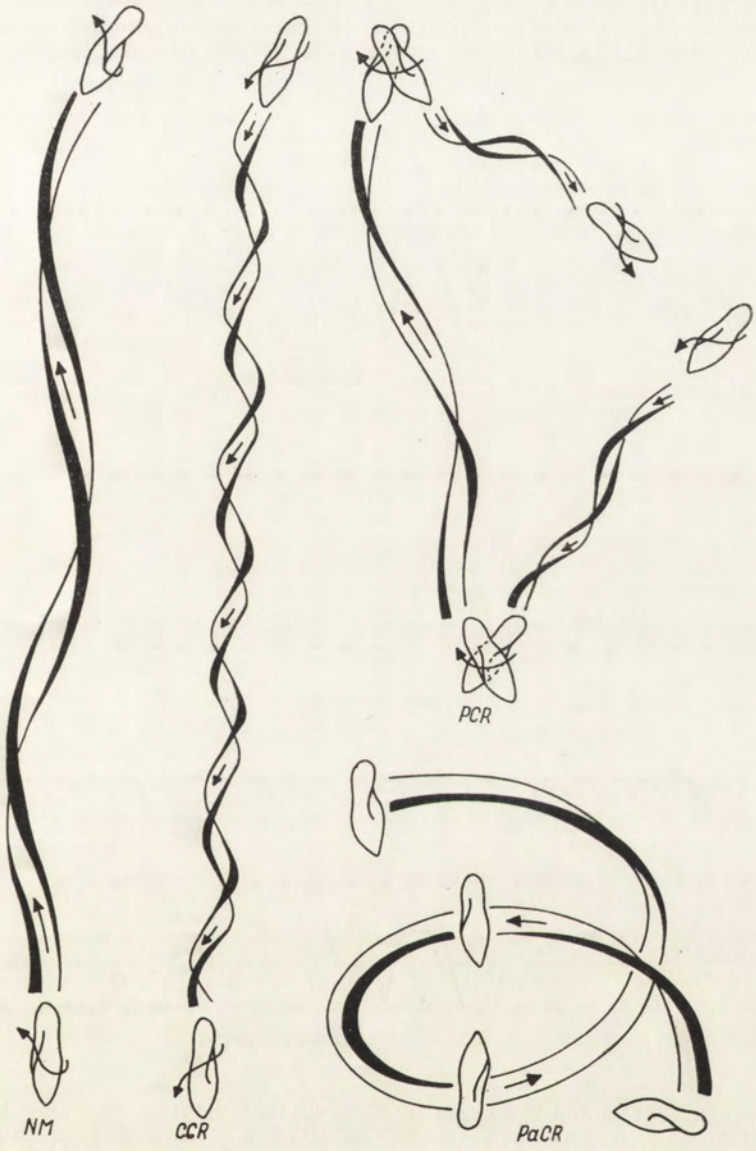


Fig. 1. The distinguished types of motor responses in *P. caudatum:* Normal movement (NM), periodic ciliary reversal (PCR), continuous ciliary reversal (CCR), partial ciliary reversal (PaCR)

Results

The results of experiments have been represented in nine diagrams (Figs. 2, 3, 4, 5, 6, 7, 8, 9, 10,) arranged in a sequence of diminishing atom weight of the cations used. Graphic illustration of the ciliate motor reactions under the action of $MgCl_2$ and LiCl have been postponed as coinciding with that of NaCl.

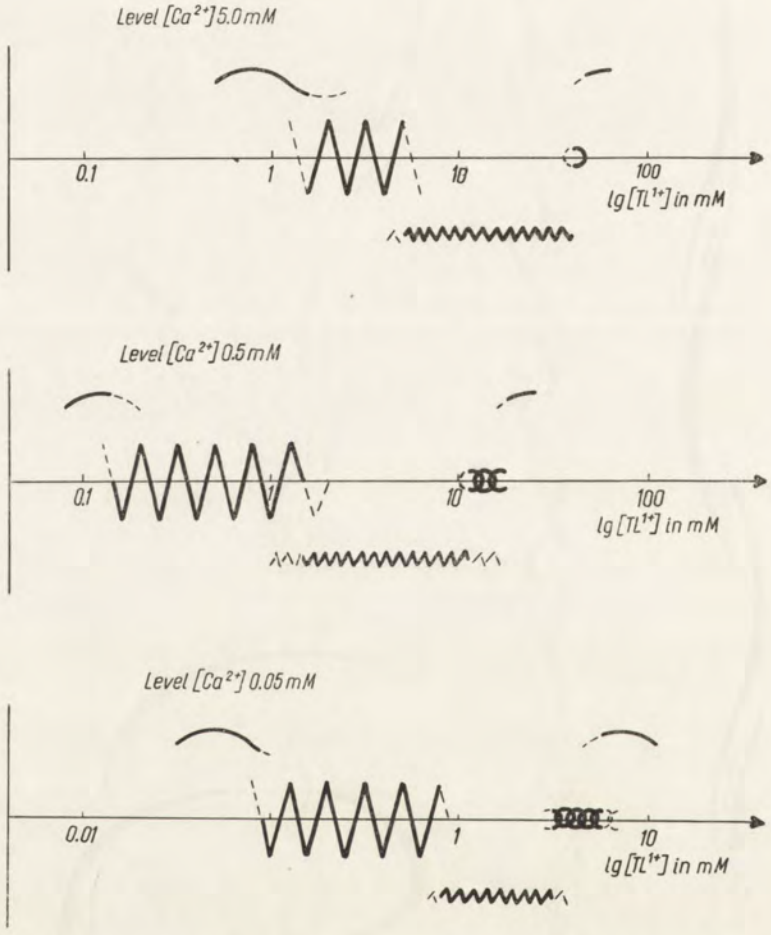


Fig. 2. Sequence of motor responses in *P. caudatum* varying according to Tl_2SO_4 concentration at three constant levels of $CaCl_2$ (5.0, 0.5, 0.05 mM) in medium. Concentration of Tl_2SO_4 in logarithmic scale is marked on the horizontal axis. The wavy line above the axis represents the normal movement, the zig-zag line transgressing the axis upwards and downwards symbolizes the periodic ciliary reversal (PCR), the notched line below the axis represents the continuous ciliary reversal (CCR), the circles on the axis symbolize the partial ciliary reversal (PaCR), continuous line—common or dominant responses, discontinuous line—non-dominant responses. Diagram represents the behaviour of ciliates in the first minute following the chemical stimulation

With the increase of Tl^{1+} ions concentration at all the three successive levels of calcium, following motor responses appear in *P. caudatum*: → PCR → CCR → PaCR → NM. This is the same sequence as that obtained by Grębecki 1965 as result of a gradual decalcification of ciliates by Ca-chelating and Ca-precipitating agents. When considering the dominating reactions, besides the Tl^{1+} ions, a similar sequence of ciliates behaviour is evoked by Ba^{2+} and Cs^{1+} ions but only at the lowest level of calcium (0.05 mM).

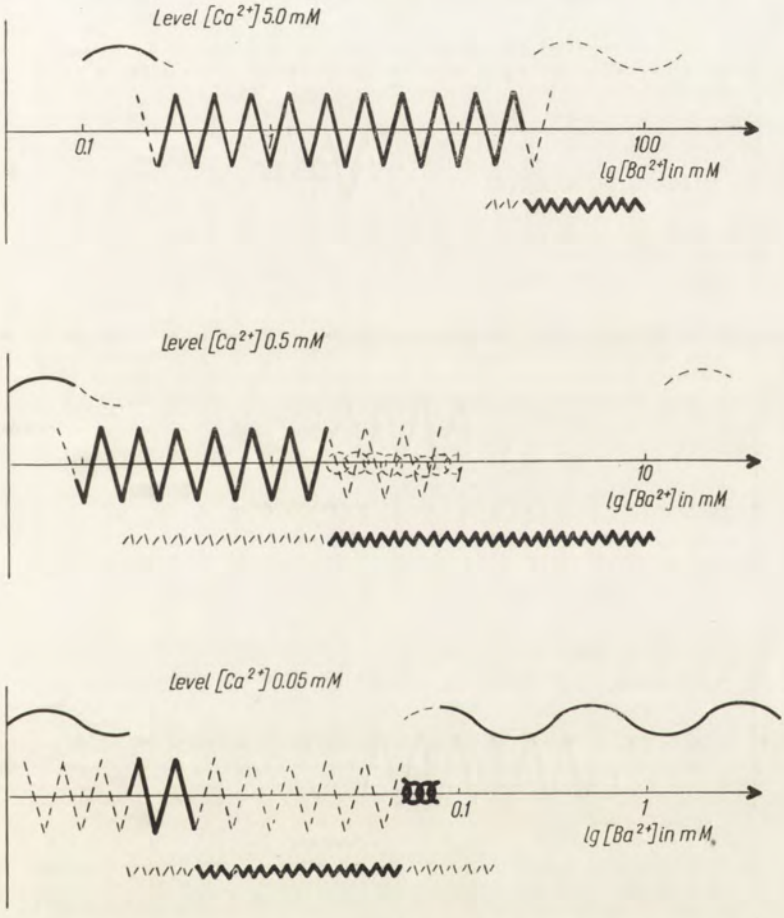


Fig. 3. Sequence of motor responses in *P. caudatum* varying according to $BaCl_2$ concentration at three constant levels of $CaCl_2$ (5.0, 0.5, 0.05 mM) in medium. Diagram is constructed in the same manner as in Fig. 2

At higher calcium concentrations (0.5 mM) the sequence for two above ions becomes reduced to: NM → PCR → CCR. At the calcium level 5.0 mM, even in the highest concentrations of Mg^{2+}, Na^{1+} and Li^{1+} — which evoke the death of ciliates within 3—5 min. — NM remains the dominating type of reaction although PCR and CCR appear as non-dominating phenomena. However at lower levels of calcium (0.5, 0.05 mM), Mg^{2+}, Na^{1+} and Li^{1+} ions evoke as well PCR as CCR as dominating reactions in the first minute following the chemical stimulation.

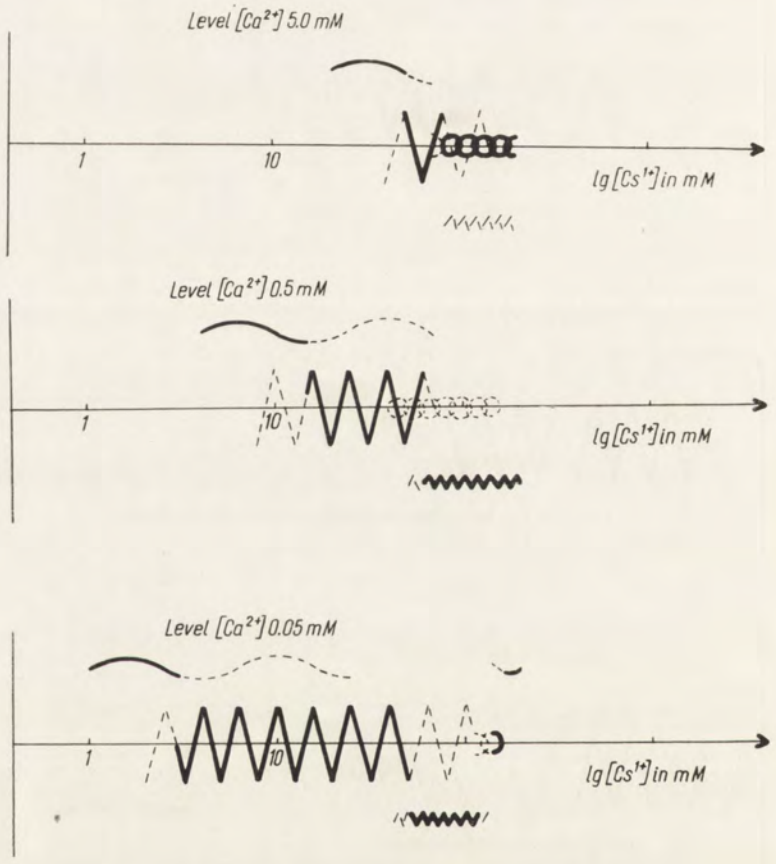


Fig. 4. Sequence of motor responses in *P. caudatum* varying according to CsCl concentration at three constant levels of $CaCl_2$ (5.0, 0.5, 0.05 mM) in medium. Diagram constructed in the same manner as in Fig. 2

The only ion which fails to change NM into another type of motor response at all the three initial Ca^{2+} levels, independently of the concentration applied — is Ca^{2+}.

It was also ascertained that under the action of some of the cations studied, the sequence NM → PCR → CCR → PaCR → NM may be not only shortened but also modified. Those modifications concern the succession of the separate motor reactions in the case of increase of the acting factor. In the Table 1

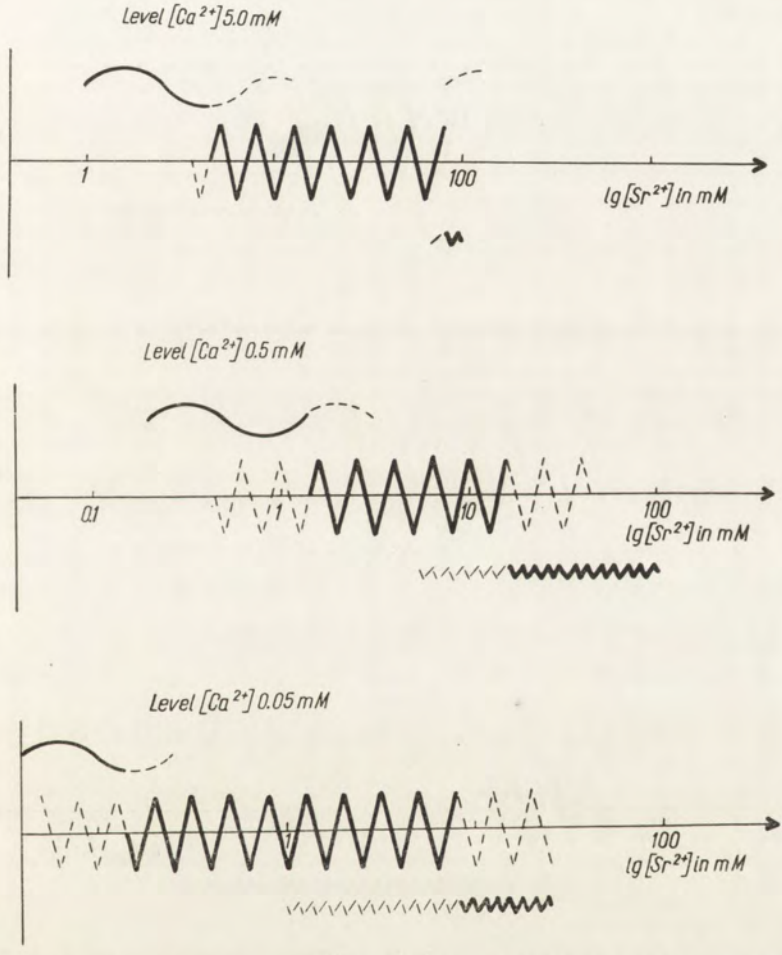
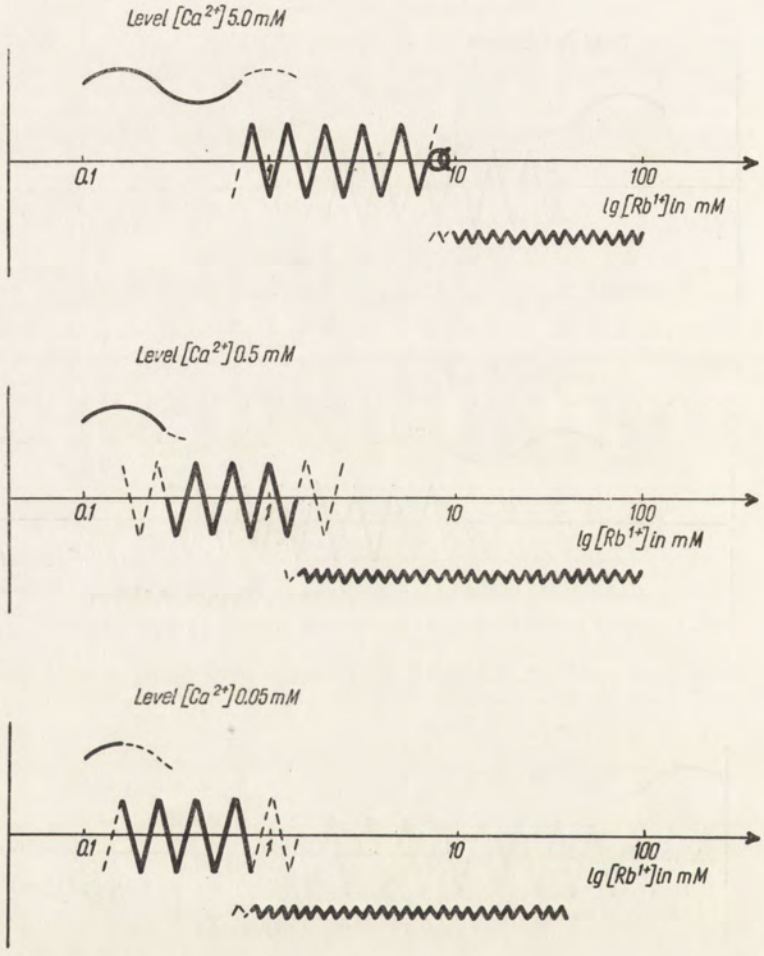


Fig. 5. Sequence of motor responses in *P. caudatum* varying according to $SrCl_2$ concentration at three constant levels of $CaCl_2$ (5.0, 0.5, 0.05 mM) in medium. Diagram constructed in the same manner as in Fig. 2

all the types of the sequence observed as well as the conditions of their occurrence are compared.

Reduction and modification of the typical sequence (NM → PCR → CCR → → PaCR → NM) were accounted for as expression of various degree of elimination of Ca ions adsorbed on the membrane of *Paramecium*, by different cations.



Ffg. 6. Sequence of motor responses in *P. caudatum* varying according to RbBr concentration at three constant levels of $CaCl_2$ (5.0, 0.5, 0.05 mM) in medium. Diagram constructed in the same manner as in Fig. 2

The decalcifyin action of the cations applied was also proved by means of control experiments. Among others, the study concerned the effect of K^{1+} 16, 32, 64 mM) on ciliates treated previously with such concentrations of Tl^{1+} (different, depending on the level of calcium) at which any type of ciliary reversal fails to appear (PCR, CCR, PaCR) but the normal movement goes on. Under those conditions potassium proved to be a fully uneffective factor. Paramecia swim then with a normal movement i.e. they behave similarly

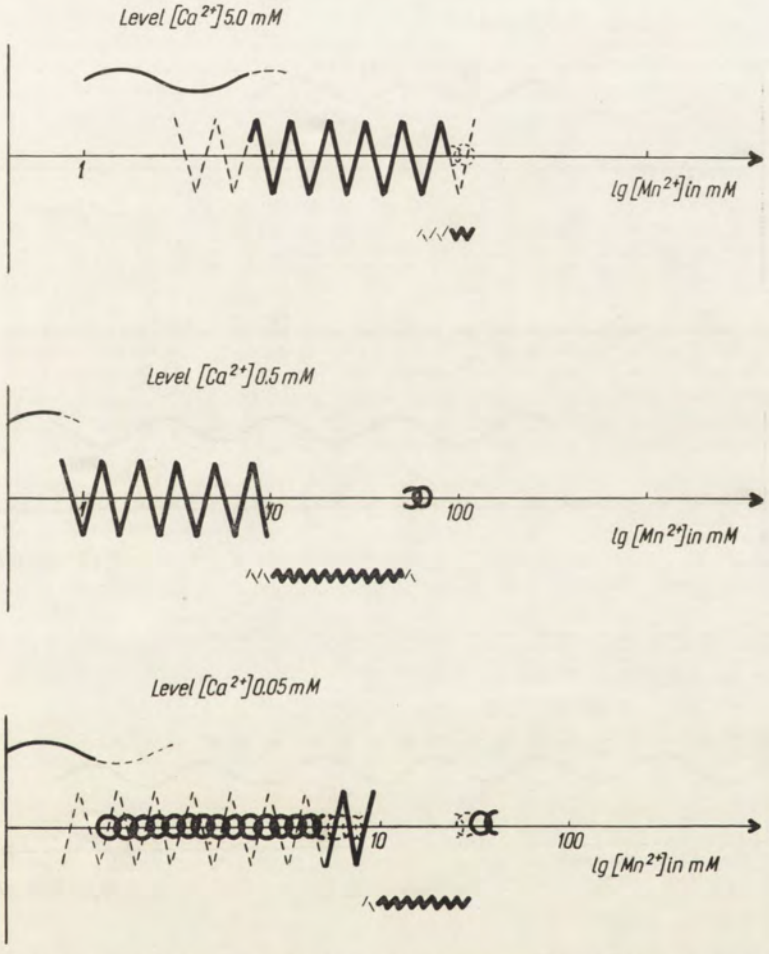


Fig. 7. Sequence of motor responses in *P. caudatum* varying according to $MnCl_2$ concentration at three constant levels of $CaCl_2$ (5.0, 0.5, 0.05 mM) in medium. Diagram constructed in the same manner as in Fig. 2

as in the media deprived of Ca^{2+} ions (K a m a d a and K i n o s i t a 1940), or under the influence of the strongly decalcifying action of EDTA (G r ę b e c k i 1965). The above fact seems to indicate that Tl^{1+} ions may decalcify the ciliate membrane as effectively as the Ca-chelating and Ca-precipitating agents do. The decalcifying capability of other cations is less effective which is expressed in the reduction and modification of the motor reaction sequence, typical for their compounds.

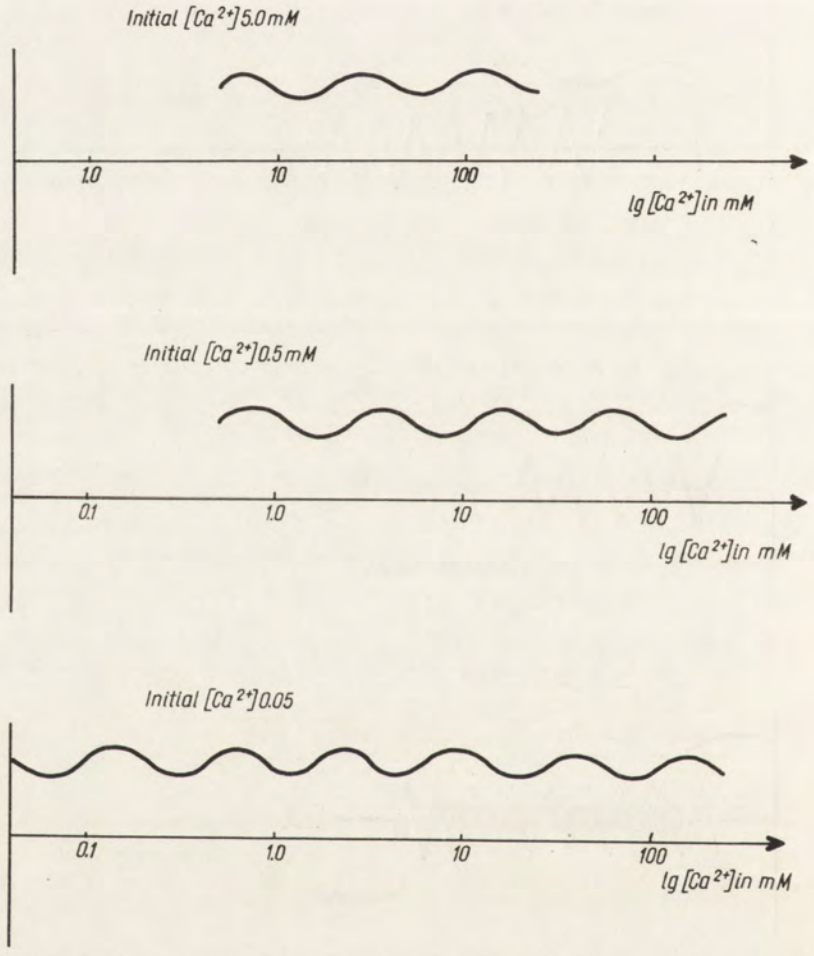


Fig. 8. Control experiment. Motor responses in *P. caudatum* varying according to $CaCl_2$ concentrations. Initial level of calcium 5.0, 0.5, 0.05 mM. Diagram constructed in the same manner as in Fig. 2

According to the Ca-content concept (G r ę b e c k i 1965), the sequence of motor responses (NM → PCR → CCR → PaCR → NM) corresponds to the gradual changes of Ca^{2+} amount which remains on the binding sites of the membrane in the ciliate. Consequently in the individuals which were decalcified by cations (after increase of Ca^{2+} ions concentration in medium), recalcification should involve an inversion of the motor reactions sequence.

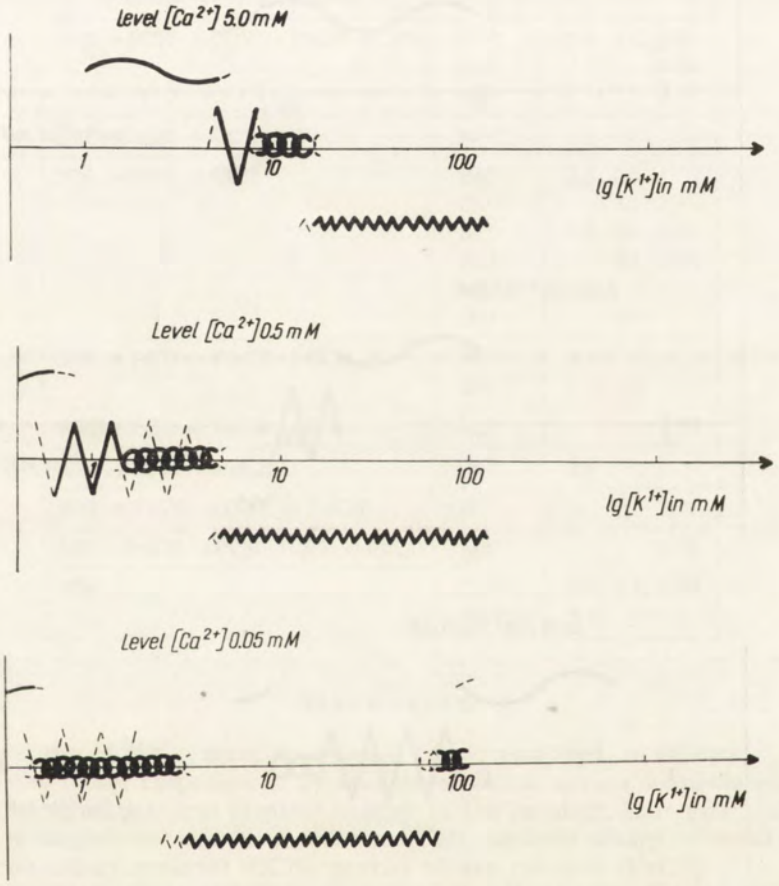


Fig. 9. Sequence of motor responses in *P. caudatum* varying according to KCl concentration at three constant levels of $CaCl_2$ (5.0, 0.5, 0.05 mM) in medium. Diagram constructed in the same manner as in Fig. 2

This postulation was proved for the ions studied with a positive result. A full sequence of motor reactions: NM → PaCR → CCR → PCR → NM, may be obtained even in highly decalcified with Tl^{1+} individuals after agradual increase of the external Ca^{2+} content.

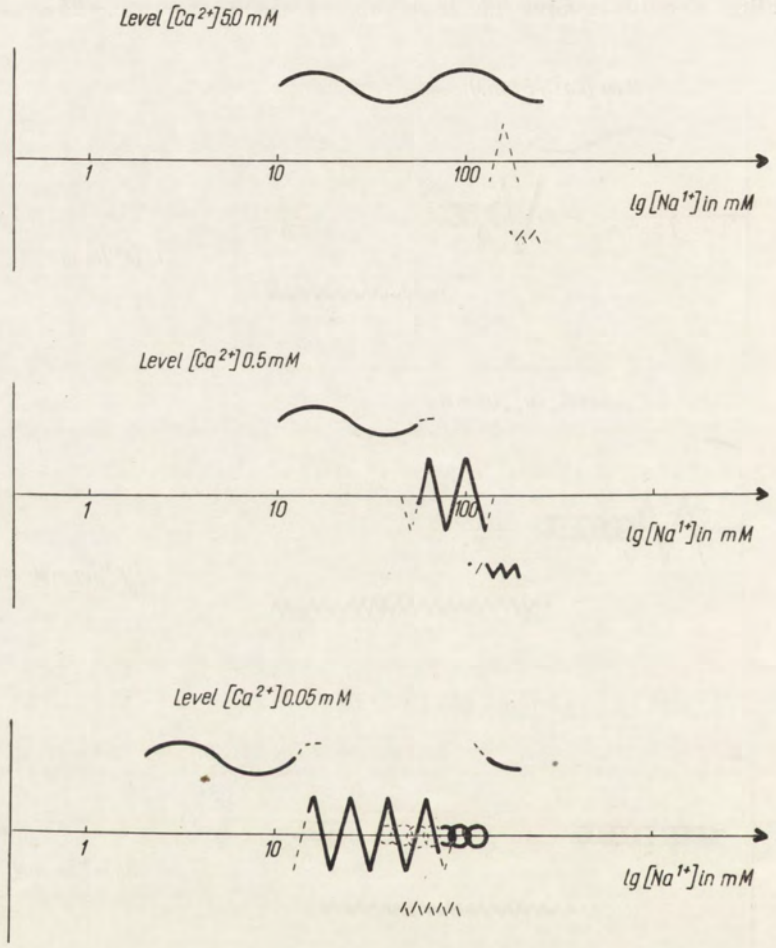


Fig. 10. Sequence of motor responses in *P. caudatum* varying according to NaCl concetration at three constant levels of $CaCl_2$ (5.0, 0.5, 0.05 mM) in medium. Diagram constructed in the same manner as in Fig. 2

Table 1

Sequence of dominatig motor responses in *P. caudatum* varying according to the concentration of some inorganic cations at three constant levels of $CaCl_2$. Behaviour of the ciliates in the first minute following the chemical stimulation

Sequence of dominating motor responses at incraesing of concentration of cations	Cations	Ca^{2+} level in mM
NM → PCR → CCR → PaCR → NM	Tl^{1+}	5.0, 0.5, 0.05
	Ba^{2+}	0.05
	Cs^{1+}	0.05
NM → PCR → CCR → PaCR	Mn^{2+}	0.5
NM → PCR → CCR	Ba^{2+}	5.0, 0.5
	Cs^{1+}	0.5
	Sr^{2+}	5.0, 0.5, 0.05
	Rb^{1+}	0.5, 0.05
	Mn^{2+}	5.0
	Na^{1+}	0.5
NM → PCR → PaCR → CCR	Rb^{1+}	5.0
	K^{1+}	5.0, 0,5
NM → PCR → PaCR → NM	Na^{1+}	0.05
NM → PCR → PaCR	Cs^{1+}	5.0
NM → PaCR → CCR → PaCR	K^{1+}	0.05
NM → PaCR → PCR → CCR → PaCR	Mn^{2+}	0.05
NM	Ca^{2+}	5.0, 0.5, 0.05
	Na^{1+}	5.0

Discussion

The results of the present study may be systematized as follows:

1. In the motor responses of *P. caudatum,* which appear immediately after addition of some uni- and bivalent cations to the medium, four essential types may be distinguished: normal movement (NM), periodic ciliary reversal (PCR), continuous ciliary reversal (CCR), partial ciliary reversal (PaCR).

2. With the increase of ion concentration the distinguished motor responses form series which depend also on the level of calcium in the medium. For the Tl^{1+} ions (at all the three levels of calcium — 5.0, 0.5, 0.05) as well as for Ba^{2+} and Cs^{1+} ions (at calcium level 0.05) this series is NM → PCR → → CCR → PaCR → NM. It is the same sequence of motor reactions as that which is involved by increase of concentration of Ca-chelating and Ca-precipitating agents (Grębecki 1965). For the other ions this series may be shortened or modified in some way. Those modifications concern mostly the change in appearance of the motor reaction of the PaCR type in the sequence.

3. Paramecia treated with Ca^{2+} — independently of the initial level of this factor in medium — fail to show any inclination to change NM into another type of motor reaction.

4. This concentration of the experimented ion which evokes the transition from one type of the motor reaction to another, increases with the rise of Ca ions concentration in medium. The level of calcium may also limit or modify the sequence of motor reactions evoked by the same factor and even — at fairly high concentrations — may inhibit the transition of NM into any other type of motor reaction. This fact may presumably account for the result of observations of O l i p h a n t (1942) who stated that Sr^{2+} and Mg^{2+} ions fail to evoke CCR in *Paramecium*.

5. Any series of reactions induced, independently of the ion applied — may be inversed by a subsequent gradual increase of Ca^{2+} in medium. E.g. paramecia treated with rather high concentrations of $Tl^{1+}$and subsequently recalcified show the following sequence of reactions: NM → PaCR → CCR → → PCR → NM, in proportion of increase of Ca^{2+} in medium. This sequence is in harmony with that observed in ciliates which were gradually recalcified after a previous strong decalcification by Ca-chelating and Ca-precipitating agents (G r ę b e c k i 1965).

6. Paramecia treated with high concentrations of Tl^{1+} at which no transition of NM into any other type of motor reaction occurs, become insusceptible to any other chemical stimulation except calcification.

The parallelism of motor responses of ciliates evoked by some inorganic cations and their reaction to Ca-chelating and Ca-precipitating agents — as stated above — suggests that their manner of action on the excitation phenomena is the same in both cases and consists in the change of the level of Ca ions adsorped in the cell membrane of *Paramecium*.

Cations eliminate the calcium ions from the cortical layer of the ciliate, possibly occupaing their binding sites. The degree of their action depends on their kind, concentration and on the Ca level in medium. The ciliate with a fully decalcified cell membrane fails to change the character of its movement being in a state of full insusceptibility to stimuli. Under such conditions only a secondary recalcification may restore its susceptibility.

According the Ca-content hypothesis (G r ę b e c k i 1965), the sequence of motor responses (NM → PCR → CCR → PaCR → NM) corresponds to the gradual changes of Ca^{2+} amount remaining on the binding sites in the membrane of *Paramecium*. The results of the present study, especially the occurrence of inversion of sequence of the motor reactions after the calcification of ciliates which have been decalcified previously, speak in favour of this concept. Only the modification of sequence — typical for decalcifying agents (in the first place the change of turn in which PaCR may appear) — remains a problem difficult for elucidation.

In the present study two essential problems concerning the behaviour of ciliates under the influence of chemical stimulation have been omitted:

1. In which way the change of Ca^{2+} ions density adsorbed on the membrane exerts influence upon the manner of action of the ciliary apparatus.

2. What is the mechanism of self-normalization of CCR and of other types of ciliary reversal occurring (except PCR evoked by Ba^{2+} and Sr^{2+}) without any changing in the external Ca^{2+} concentration.

The solution of these problems requires further experimental study.

Summary

Motor responses of *P. caudatum* which manifest immediately after addition of inorganic uni- and bivalent cations to medium, may be ranged in four essential types: NM, PCR, CCR, PaCR. These distinguished reaction types appear in a definite sequence together with the gradual rise of the ion concentration. The motor reactions may form series NM → PCR → CCR → → PaCR → NM which depends on the kind of cation and on the level of calcium in medium. Those series are the same as the sequence produced by the Ca-chelating and Ca-precipitating agents. The series may be reduced e.g. NM → PCR → CCR, or modified. The modifications consist in the first place in the changes of PaCR consecution.

Each series of motor responses, independently of the ion, may be inversed by a gradual rise of Ca^{2+} in medium. Ciliates treated with high concentrations of Tl^{1+} show no change of NM into another type of motor reaction and become insusceptible to the action of other cations except Ca^{2+}.

The results permit to postulate that all the motor reactions in *Paramecium* evoked by the chemical stimulation consist in one common mechanism of Ca ions desorption from the ciliate membrane. Consequently the motor reactions would correspond to the gradual changes of amount of Ca^{2+} remaining on the binding sites in the membrane of *Paramecium* (Ca-content hypothesis — Grębecki 1965).

STRESZCZENIE

Reakcje ruchowe *P. caudatum*, występujące bezpośrednio po dodaniu do środowiska jedno- i dwuwartościowych kationów nieorganicznych można sprowadzić do czterech zasadniczych typów: NM, PCR, CCR, PaCR. Wraz ze wzrostem stężenia jonu, wyróżnione typy reakcji pojawiają się w określonej kolejności. W zależności od rodzaju kationu i poziomu wapnia w środowisku, reakcje ruchowe mogą tworzyć serię NM → PCR → CCR → PaCR → NM (identyczną z sekwencją jaką dają czynniki chelujące i wytrącające wapń), bądź jej postać skróconą (np. NM → PCR → CCR) lub zmodyfikowaną.

Modyfikacje te sprowadzają się przede wszystkim do zmiany kolejności pojawiania się PaCR.

Każda seria reakcji ruchowych, niezależnie od jonu może być odwrócona przez stopniowe zwiększanie Ca^{2+} w środowisku.

Orzęski poddane działaniu wysokich stężeń Tl^{1+} przy których nie zachodzi zmiana NM w inny typ reakcji ruchowej, stają się niewrażliwe na wpływ innych kationów z wyjątkiem Ca^{2+}.

Uzyskane wyniki, pozwalają sądzić, że wszystkie reakcje ruchowe *Paramecium*, wywoływane przez stymulację chemiczną, polegają na jednym wspólnym mechanizmie — desorpcji jonów wapnia z błony orzęsków. Tym samym, kolejne reakcje ruchowe odpowiadałyby stopniowo zmniejszającemu się poziomowi jonów wapnia zaadsorbowanych na błonie *Paramecium* (Grębecki 1965).

REFERENCES

Dryl S. 1958: Photographic registration of movement of *Protozoa*. Bull. Acad. pol. Sci., Cl. II 6, 429—430.

Grębecki A. 1965: Role of Ca^{2+} ions in the excitability of protozoan cell. Decalcification, recalcification, and the ciliary reversal in *Paramecium caudatum*. Acta Protozool. 3, 275—289.

Jahn T. L. 1962: The mechanism of ciliary movement. II. Ion antagonism and ciliary reversal. J. cell. comp. Physiol. 60, 217—228.

Kamada T. 1938: Intracellular calcium and ciliary reversal in *Paramecium*. Proc. imp. Acad. Japan, Tokyo 14, 260—262.

Kamada T. 1940: Ciliary reversal of *Paramecium*. Proc. imp. Acad. Japan, Tokyo 16, 241—247.

Kamada T. and Kinosita H. 1940: Calcium-potassium factor in ciliary reversal of *Paramecium*. Proc. imp. Acad. Japan, Tokyo 16, 125—130.

Oliphant J. F. 1942: Reversal of ciliary action in *Paramecium* induced by chemicals. Physiol. Zool. 15, 443—452.

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Ciliary reversal in *Paramecium caudatum* in relation to external pH

Rewersja rzęskowa u *Paramecium caudatum* w zależności od zewnętrznego pH

The physico-chemical properties and reactivity of many organic substances, particulary proteins, depends on the pH. Among others the changes in the hydrogen ion concentration, exert an essential influence on the adsorption phenomenon. For example, an increase of pH from neutral to alkalic one, raises the number of anion binding sites in the same proteins and increases the uptake of free cations from the medium (for review see: Ling 1962).

Grębecki and Kuźnicki 1963 have indicated, that toxicity of inorganic cations in *Paramecium caudatum* rises with the alkalization of medium and falls with its acidification, while the toxicity of anions seems to be independent from pH. Considering all those data, it may be assumed that changes in external pH alterate the adsorption conditions in the membrane of ciliates in similar way as in some proteins.

Moreover, the continuous ciliary reversal (CCR) involved in *Paramecium* by chemical stimulation was investigated up to now under such conditions and analyzed with the presumption made, that the number of the active groups in the surface layer of ciliates, binding cations, remains constant during experiment (Jahn 1962, Grębecki 1964, 1965, Kuźnicki 1966). In this paper the results of the study on the dependence of the time of continuous ciliary reversal (CCR), evoked in *P. caudatum* by KCl and Tl_2SO_4, on pH of the medium will be reported. Information on this subject may throw some light upon behaviour of the ciliates membrane and mechanism of self-normalization of CCR. Some data relative to effects of external pH on the cations induced ciliary reversal have been presented earlier in short abstract (Kuźnicki 1965).

Results

Experiments were performed on *Paramecium caudatum* grown in the milk-fed culture. Ciliates taken from thimogotactic ring were rinsed four times in series of buffers until the initial medium became diluted 10 000 times. Most frequently Tris-HCl (standard solution diluted 1 : 10) was used, providing a constant level of 0.5 mM $CaCl_2$ in the medium.

For decreasing pH, acid potassium phtalate-NaOH buffer (standard solution diluted 1 : 20) or the mixture of 10 mM H_3BO_3+HCI were applied, always at

the constans level of 0.5 mM $CaCl_2$ in the medium. Prior to experiment ciliates were kept in buffer for various periods of time from 15 min. up to 24 hrs. The buffer applied evoked in paramecia neither lethal changes nor disturbances in movement during $>$ 24 hrs. KCl and Tl_2SO_4 were also diluted in buffer. Observations of CCR duration were carried out on mass samples, containing not more than 100 ciliates per 1 ml., in concentration: 8 mM or 16 mM KCl, and 2 mM or 4 mM Tl_2SO_4. The duration of CCR was recorded in the same

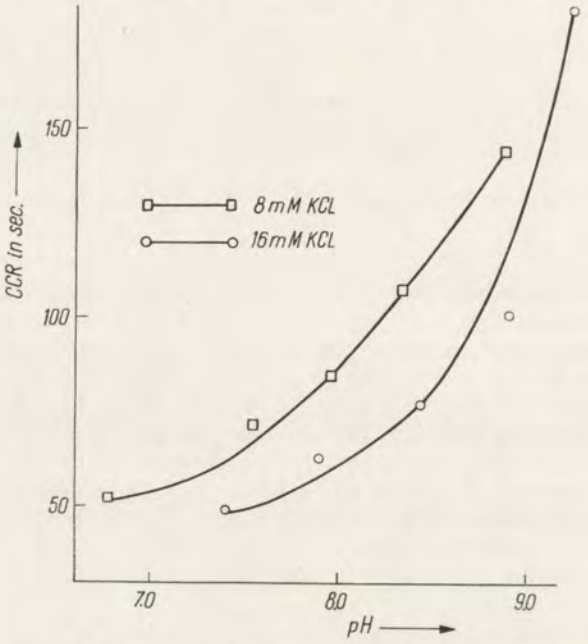

Fig. 1. The dependence of continuous ciliary reversal (CCR) evoked in *Paramecium caudatum* by 8 mM and 16 mM KCl, on the changes in pH of medium, involved by Tris-HCl buffer (standard solution diluted 1 : 10) + 0.5 mM $CaCl_2$. Time of keeping the ciliates in buffer: 8 mM KCl — 1 hrs., 16 mM — 24hrs. Each point — average of 20 measurements

manner as described G r ę b e c k i 1964. The pH value was controlled with potentiometr in each experiment. The investigation was carried out at 21 $\pm$ 1°C.

The results of experiments involving an increase of pH are shown in the diagrams. The curves illustrate the dependence of CCR duration evoked by K^{1+} (Fig. 1) and Tl^{1+} (Fig. 2), on the changes in pH involved by application of Tris-HCl buffer. In the case of both cations the expected relation is distinct. Rise of pH involves a prolongation of CCR, while a decrease shortens its duration. The increase of pH results also in a fall of the threshold concentration of K^{1+} and Tl^{1+} ions, evoking CCR. The time of keeping the ciliates in the buffer has no the manifestation of this regularity, at the range studied.

The dependence reported above holds true for the acidic range as well, but it appears not so regular as in the alkalic one, and requires further experimen-

tal study. However in general it may be ascertained that the decrease of pH shortens the duration of CCR up till extinguishing the possibility of evoking it. At the pH below 4.9, independently of the composition of the buffer, the continuous ciliary reversal fails to occur at all, even if 16 mM KCl and 4 mM Tl_2SO_4 are applied.

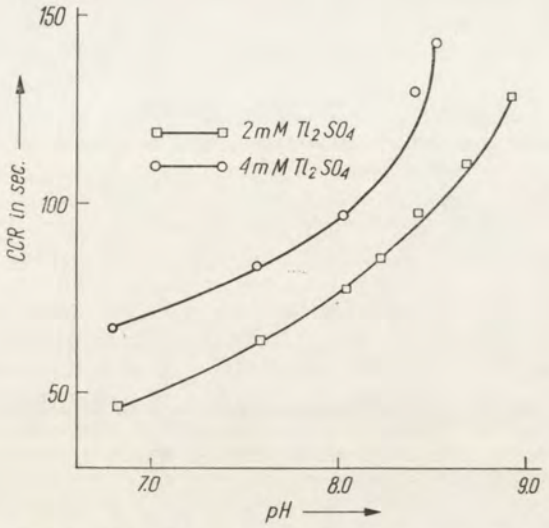

Fig. 2. The dependence of continuous ciliary reversal (CCR) evoked in *P. caudatum* by 2 mM and 4 mM Tl_2SO_4, on the changes in pH of medium involved by Tris-HCl buffer (standard solution diluted 1 : 10) + 0.5 mM $CaCl_2$. Time of keeping the ciliates in buffer: 2 mM Tl_2SO_4 — 24 hrs., 4 mM Tl_2SO_4 — 30 min. Each point — average of 20 measurementes

Paramecia rinsed and kept only in 0.5 mM $CaCl_2$ and treated with KCl and Tl_2SO_4 solutions prepared at the same calcium level were used as the control. Under these conditions the time of ciliary reversal corresponded to CCR duration involved by the same K^{1+} and Tl^{1+} concentration in buffer of the value 7.1—7.5.

Concluding remarks

To my knowledge the effect of external pH on the cation-induced CCR in *P. caudatum* has not been analyzed as yet. Nevertheless the results of the present study correspond fairly to the following earlier observations:

1. At a constant respectively low, concentration of $SrCl_2$ alkalization of medium evokes in ciliates the change of normal movement for periodic ciliary reversal (Eisenberg-Hamburg 1932).

2. The hydroxides of the alkali metals are in general more effective in inducing CCR in paramecia than are their salts (Oliphant 1942).

3. Toxicity of inorganic cations in *P. caudatum* increases with the alkaliza-

tion of medium and decreases with its acidification (Grębecki and Kuźnicki 1963).

4. Rise of pH of medium induces an increase of the membrane potential in *Paramecium,* while the fall of pH involves a opposite effect (Kinosita, Dryl and Naitoh 1964).

Considering all those facts cited above and the results presented in this paper it may be assumed:

1. The changes of pH of medium exert the essential influence on the physico-chemical state and properties of the membrane in *P. caudatum.* The rise of pH augments the number anion of binding sites in the surface layer of ciliate and increases the uptake of cations, while the fall of the medium pH reduces the number of the active anionic groups and decreases the adsorption of cations.

2. Duration of CCR depends on the adsorption conditions in the membrane. Increase of the number of the anion binding sites in surface layer of ciliates involves prolongation of the CCR induced by cations, while decrease in number of active anion groups shortens the time of CCR up till extinguishing the possibility of evoking it. In the limits of viability (up to pH 4.6) even an abrupt fall of pH fails to evoke CCR. Consequently, it may be postulated that decalcification induced by reducing the number of anion binding sites in the membrane has no effect on the motor response of *Paramecium,* even if the content of Ca^{2+} adsorbed falls in such a degree which involves the insuceptibility of cilites to chemical stimuli.

Summary

In *Paramecium caudatum* the dependence of the duration of continuous ciliary reversal (CCR), evoked by cations K^{1+} and Tl^{1+}, on the pH of the medium was investigated. Raising of pH results in prolongation of CCR, while a decrease in pH shortens its duration. The increase of pH causes also fall of the threshold of the concentration K^{1+} and Tl^{1+} ions, evoking CCR. Those data are interpreted as effect of external pH on alteration of the adsorption conditions in the surface layer of the ciliate. Probably increase of the number of the binding sites, in *Paramecium* membrane involved prolongation of the CCR, while their decrease in number shortens the time of CCR up till extinguishing the possibility of evoking it.

STRESZCZENIE

Badano wpływ jaki wywiera pH środowiska na czas trwania rewersji rzęskowej, (CCR), wywołanej u *Paramecium caudatum* przez jony K^{1+} i Tl^{1+}. Wzrost pH wydłuża czas rewersji rzęskowej, podczas gdy obniżenie pH czas ten skraca. Wraz ze wzrostem pH obniża się również progowe stężenie, przy którym jony K^{1+} i Tl^{1+} wywołują CCR.

Wyniki te zostały zinterpretowane jako przejaw wpływu pH środowiska na zmianę warunków adsorpcji w warstwie powierzchniowej orzęska. Prawdopodobnie wzrost liczby miejsc adsorpcji na błonie *Paramecium* powoduje wydłużanie się czasu rewersji rzęskowej, podczas gdy zmniejszanie tej liczby skraca czas trwania CCR, aż do zniesienia możliwości jej wywołania.

REFERENCES

Eisenberg-Hamburg E. 1932: Einfluss der Sr-Salze auf die Bewegung von *Paramecium caudatum*. Die Rolle des Ca und der Konzentrationen der Wasser stoffionen. Arch. Protistenk. 77, 108—124.

Grębecki A. 1964: Rôle des ions K^+ et Ca^+ dans l'excitabilité de la cellule protozoaire. Équilibrement des ions antagonistes. Acta Protozool. 2, 69—79.

Grębecki A. 1965: Role of Ca^{+2} ions in the excitability of protozoan cell. Decalcification, recalcification, and the ciliary reversal in *Paramecium caudatum*. Acta Protozool. 3, 275—289.

Grębecki A. and Kuźnicki L. 1963: The influence of external pH on the toxicity of inorganic ions for *Paramecium caudatum*. Acta Protozool. 1, 157—164.

Jahn T. L. 1962: The mechanism of ciliary movement. II. Ion antagonism and ciliary reversal. J. cell. comp. Physiol. 60, 217—228.

Kinosita H., Dryl S. and Naitoh Y. 1964: Relation between the magnitude of membrane potential and ciliary activity in *Paramecium*. J. Fac. Sci. Tokyo Univ. 10, 303—309.

Kuźnicki L. 1965: Effect of external pH on cation induced ciliary reversal in *Paramecium caudatum*. Progress in Protozoology, Abstr. Second int. Conf. Protozool., London 1965, Excerpta med. Int. Congr. ser. No 91, abstr. 300.

Kuźnicki L. 1965: Role of Ca^{2+} ions in the excitability of protozoan cell. Calcium factor in the ciliary reversal induced by inorganic cations in *Paramecium caudatum*. Acta Protozool. 4, 241—256.

Ling G. N. 1962: A physical theory of the living state: the association-induction hypothesis. Blaisdell Publishing Company, New York-London.

Oliphant J. F. 1942: Reversal of ciliary action in *Paramecium* induced by chemical. Physiol. Zool. 15, 443—452.

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Inversion of spiralling of *Paramecium aurelia* after homologous antiserum treatment

Inwersja spiralizacji *Paramecium aurelia* pod wpływem homologicznej surowicy odpornościowej

Párducz 1962 stated the characteristic effect of low $NiSO_4$ concentration upon *Paramecium multimicronucleatum*, the change of spiralling direction from the normal forward left spiralling to the forward right spiralling. This manner of swiming persists till the moment of the immobilization of the ciliate.

This observation was fully confirmed in *P. caudatum* and *P. aurelia* by Grębecki, Kuźnicki and Mikołajczyk 1966 a. As follows from the study of Alverdes 1922, Ludwig und Schlicksupp 1951, and Grębecki, Kuźnicki and Mikołajczyk 1966 b, inversion of spiralling may be evoked in both species by a raise of medium viscosity. Comparison of these facts suggests that in *P. caudatum* and in *P. aurelia*, inversion of spiralling direction is not a specific response to a definite factor but a type of movement which appears under conditions complicating the work of the ciliary apparatus. In the present study this hypothesis has been proved experimentaly by analysis of the motor respose of *P. aurelia* to homologous antiserum evoking the immobilization of ciliates.

Material and methods

Material for the study was the serotype 51 A of *Paramecium aurelia* (KK, free of "kappa"), syngen 4. Serotype 51 B served as a control.

The monobacterial culture was carried out and antisera were produced according the Sonneborn's method, modified by Sikora 1966. The culture and the whole experimental procedure were carried out at $20 \pm 1°C$.

Antiserum was diluted in 4 mM Michaelis phosphate buffer pH 6.8, in which K_2HPO_4 was substituted by $Na_2HPO_4 + 1$ mM $CaCl_2$. Prior to application the serum was dialized in this buffer for 48 hrs (5 ml of serum in 3 l of buffer).

For registration of the ciliates paths, the method of photomacrographic registration (D r y l 1958) was adapted by executing continous exposures of ciliates.

Experiments were performed in the following way:

1. Paramecia were densified by their negative geotaxy.
2. Filtered through cotton wool for eliminating the detritus.
3. Rinsed (using the Wattman's filter Nr. 1) in the buffer used for diluting the antiserum. Paramecia were used for experiments at least 1 hr after being rinsed in the buffer. The buffer applied evoked neither lethal changes nor disturbances in movement during 24 hrs.
4. 1 ml of medium containing 800—1000 individuals was mixed with 1 ml of homologous antiserum in concentration twice as high as needed in the experiment. pH was controlled in the course of experiment.
5. The first recordings of movement were executed 1 min. after addition of the homologous antiserum, the subsequent — at 15 sec. intervals. The time of exposure was 5 sec.

The behaviour of *Paramecium aurelia* after the action of homologous and nonhomologous antisera was examined in concentrations: 1 : 50 and 1 : 100. The immobilization titre of the homologous antiserum was 1 : 800, determined according to B e a l e 1948. Observations were also carried out placing the two different serotypes in one homologous antiserum simultaneously.

Results

Placed in a solution of homologous antiserum *P. aurelia* manifests following typical motor responses:

1. Normal forward left spiralling movement (FLS) with avoidings which cause the change of swiming direction (Pl. I 1, Pl. II 7—8).
2. Continuous ciliary reversal (CCR) lasting 2—15 sec. (Pl. I 2) as example.
3. Forward right spiralling (FRS). Individuals which leave CCR began a FRS. From that moment on till the immobilization, FRS is the only form of forward movement of ciliates. Avoidings continue to occur initially but their frequency diminishes graudally (Pl. I 3—4). FRS may be also transformed into CCR and CCR into FRS (Pl. II 9—10).
4. Immobilization (Imm.). In course of time after introduction of the ciliates into the solution of the homologous antiserum, the rate of their swiming decreases gradually till their complete immobilization occurs. The criterion of immoblization, was the state in which the ciliate cannot perform a forward movement exceeding its body length. At this stage it may however perform some short-lasting "vibrations", similary as in the case of the nickel immobilization ("winces" as denoted by K u ż n i c k i 1963). The photomacrographic registration of this type of movement produces images in the shape of „V" and „Y" after single reaction, and of a „star" after its multiple repetitions (Pl. I 6, Pl. II 12).

After exposure of *P. aurelia* to homologous antiserum in concentration 1 : 50 avoiding reactions appear already after a few seconds, notwithstanding the fact that normal FLS continues. 30 seconds later ciliates show the CCR. The majority of individuals enter this phase after 1 min. nad 15 sec. At this stage CCR lasts usually 10 sec. Subsequently FRS follows. Both types of

relation (CCR and FRS) occur; however they alternate till immobilization takes place.

In a solution of homologous antiserum diluted 1 : 100, avoidings appear after 30 sec. CCR appears later (after 1 min. and 30 sec.) and lasts no longer than several seconds. Therafter, the individuals which passed from FLS to CCR leave it for FRS. In this concentration of antiserum CCR inclines to fall whereas FRS still appear. Prior to immobilization all the individuals show the movement of the FRS type, with short-lasting "avoidings" (Pl. I 4—5, Pl. II 11).

The sequence of the types of the motor responses in *P. aurelia* as described above has been illustrated in Fig. 1. Besides the general similitudes of behaviour, the concentration of the homologous antiserum modifies the duration of different types of motor responses.

Two types of control experiments were carried out. In the first one the paths and character of spiralling of *P. aurelia* 51 A in the buffer solution

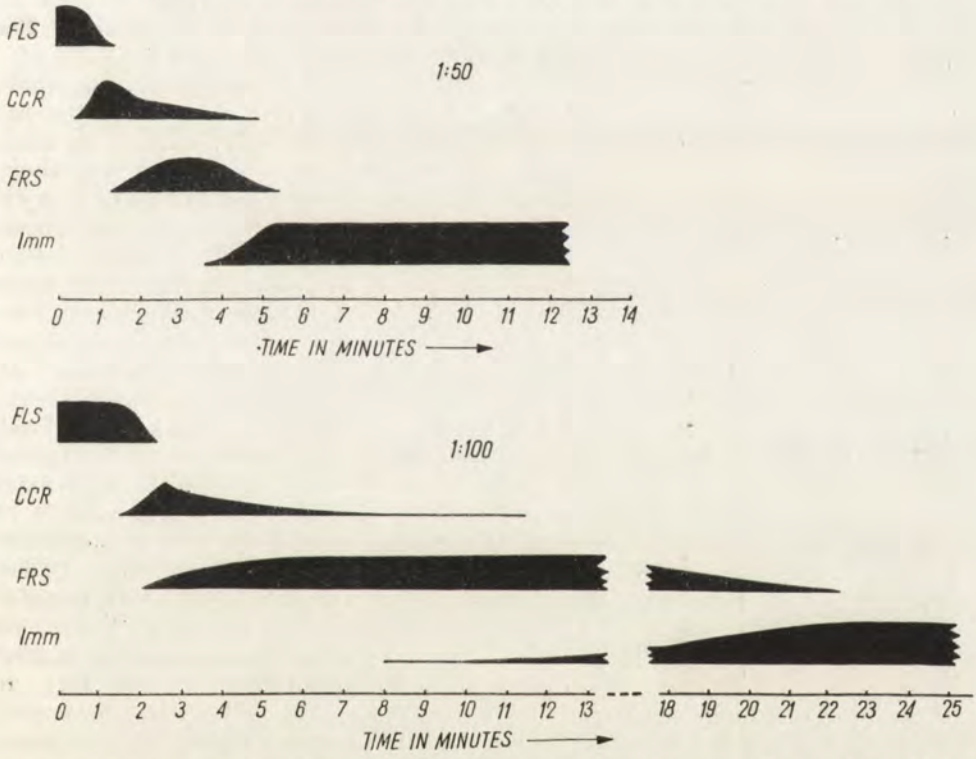


Fig. 1. Diagram of typical motor responses of *Paramecium aurelia* serotype 51 A, induced by homologous antiserum, diluted 1:50 and 1:100 in Michaelis phosphate buffer (modified), pH 6.8. The forward left spiralling (FLS), the continuous ciliary reversal (CCR), the forward right spiralling (FRS), the immobilization (Imm.). Graphic illustration of two types of experiments based on evaluation of consecutive photograms taken at 15 sec. intervals. Thickness of each stripe vizualizes the intensity of occurrence of definite type of reaction in percentage. In the case when the same individual performed two movements of different types, it was included to that type of reaction in which it covered a longer path

applied (Pl. III 13) were compared with the manner of swimming of *P. aurellia* 51 A placed in nonhomologous anti-51 B serum (Pl. III 14). Alteration in tthe movement and inclination to inverse spiralling were not observed.

In another type of control experiments, the character of movement was examined in *P. aurelia* 51 B placed together with 51 A in a solution of antti--51 A serum (Pl. III 15—18). Even 20—30 min. after the onset of experimemt, paramecia of the serotype 51 B show FLS.

Discussion

The results of investigation presented above fully support the hypothessis put forward in the introduction. In *P. aurelia* the inversion of spiralling from the FLS into FRS takes place under the action of the homologous antiserum. This manner of swimming persists as a unic form of forward movement till the moment of immobilization. This fact escaped the attention of protozoologists although another types of motor responses as rapid changes of the movement direction (generaly determined as avoiding reaction) or tlhe ciliary reversal had already been observed previously (Beale 1954).

From the point of view of the problems under consideration, the most important seems to be the parallelism of the motor responses under the influence of homologous antiserum and the behaviour of *P. aurelia* in solutions of nickel salts which evoke immobilization in a comparatively short time i.e. in several minutes (Grębecki, Kuźnicki and Mikołajczyk 1966 a). In both cases the change of FLS for FRS occurs through the phase of CCR. Since the moment of FRS appearence, it is the only manner of forward swimming until complete immobilization (in solutions of nickel sallts a full abolishment of rotation may occur before stoppage of movement). Paramecia immobilized by homologous antiserum, similarly as those immobilized by nickel ions perform from time to time a characteristic "vibrations" at a few second long intervals. In both cases this reaction consists in short-lasting ciliary reversal (Kuźnicki 1963, Grębecki, Kuźnicki amd Mikołajczyk 1966 a), which may be followed by the return to the initiial position, or the ciliate may shift under an angle in relation to its body axiis, but always without any possibility of effective displacement of the body.

Under conditions of experiment, the change of FLS for FRS through tlhe phase of CCR is characteristic for the action of homologous antiserum. Undter the influece of nickel ions the change of FLS for FRS may occur directlly without CCR. This type of reaction was observed by Párducz 1962 iin *P. multimicronucleatum,* in a medium deprived of other ions except $NiSO_4$. Grębecki, Kuźnicki and Mikałajczyk 1966 a showed that iin *P. aurelia* and in *P. caudatum* the change from FLS to FRS by movememt along arch (loop) is typical for the conditions in which immobilization induced by nickel ions fails to occur intensly. Except this difference, the genteral similitude of ciliate behaviour in both cases of immobilization is as fær convergent that it allows to put forward some general conclusions.

FRS seems to be a form of movement which is independent of the nature of factor evoking it and appears under conditions in which the work of the ciliary apparatus is in some way hampered. These conclusion is supported by the general inversion of spiralling after the transfer of *P. aurelia* and *P. caudatum* into a medium of a raised viscosity (Alverdes 1923,

Ludwig und Schlicksupp 1951, Grębecki, Kuźnicki and Mikołajczyk 1966 b). A logical consequence of those facts is the postulation that FRS, which conforms with the direction of twist of the body, is hydrodynamically a more effective movement than FLS. Consequently its appearance should be recognized as a stereotypical kind of response to difficulty in the forward movement, as postulated by Grębecki, Kuźnicki and Mikołajczyk 1966 a. The definition "stereotypical" is applied for stressing that FRS occurs and persists as a general phenomenon in folowing cases: 1. increased viscosity of medium (the mechanical character of the interfering obstacle distributed regularly over the whole ciliature), 2. superficial action (irregular sticking together of cilia caused by homologous antiserum), 3. nickel immobilization (inhibition effect of chemical nature).

The postulated effectiveness of the motor responses in *P. aurelia* FLS < FRS fails to determine the character of the physiological mechanisms which condition the occurrence of those phenomena.

Summary

In *Paramecium aurelia,* prior to immobilization by homologous antiserum the inversion of spiralling from the forward left (FLS) into the forward right (FRS) one appears as a general phenomenon. The change of FLS for FRS passes through a phase of ciliary reversal (CCR). Since the onset of spiralling inversion till the moment of the ciliate stopping, FRS remains the only mode of the ciliate forward movement. These observations support the hypothesis that FRS — as more effective hydrodynamically than FLS — is type of motor response in *P. aurelia* which is independent of the nature of evoking factor and appears in conditions of hindered action of the ciliary apparatus.

STRESZCZENIE

Pod wpływem homologicznej surowicy odpornościowej wywołującej immobilizację, pojawia się u *P. aurelia* jako zjawisko powszechne inwersja spiralizacji z lewoskrętnej (FLS) w prawoskrętną (FRS). Przejście FLS w FRS zachodzi przez fazę rewersji rzęskowej (CCR). Od chwili wystąpienia inwersji spiralizacji aż do momentu zatrzymania się orzęska, FRS pozostaje jedynym sposobem poruszania się pierwotniaka do przodu. Obserwacje te potwierdzają hipotezę, że FRS jako efektywniejszy hydrodynamicznie niż FLS, jest typem reakcji ruchowej *P. aurelia,* który niezależnie od czynnika wywołującego, pojawia się w warunkach utrudnionej pracy aparatu rzęskowego.

REFERENCES

Alverdes F. 1922: Studien an Infusorien über Flimmerbewegung, Lokomotion und Reizbeantwortung. Arb. exp. Biol., Berlin. 3. 1—133.

Beale G. H. 1948: The process of transformation of antigenic type in *P. aurelia,* variety 4. Proc. nat. Acad. Sci., Wash., 34, 418—423.

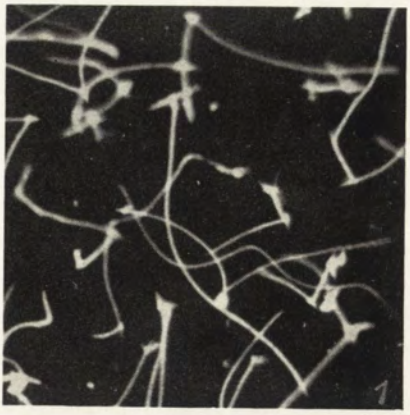
Beale G. H. 1954: The genetics of *Paramecium aurelia.* Cambridge University Press, Cambridge.

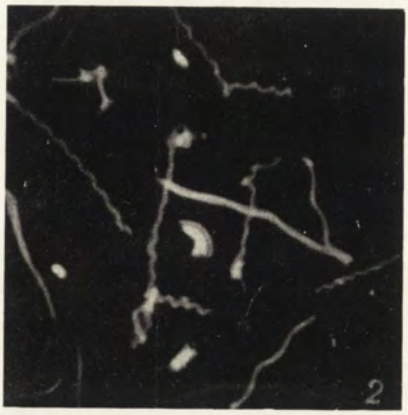
Dryl S. 1958: Photographic registration of movement of Protozoa. Bull. Acad.. pol. Sci., Cl. II. 6, 429—430.

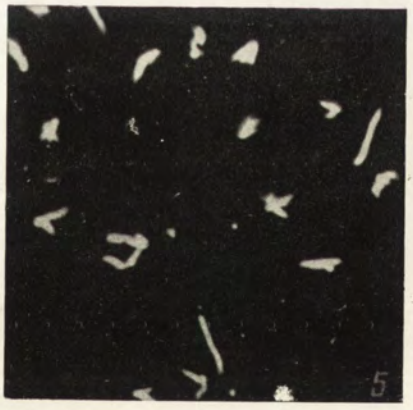
Grębecki A., Kuźnicki L., Mikołajczyk E. 1966 a: Right spiralling induced in *Paramecium* by Ni ions and the hydrodynamics of the spiral movement. Acta Protozool. in press.
Grębecki A., Kuźnicki L., Mikołajczyk E. 1966 b: Some observations on the inversion of spiralling in *Paramecium caudatum*. Acta Protozool. in press.
Kuźnicki L. 1963: Reversible immobilization of *Paramecium caudatum* evoked by nickel ions. Acta Protozool. I, 301—312.
Ludwig W. und Schlicksupp E. 1951: Der Windungssinn der Schraubenbahnen von Mikroorganismen und Larven als phylogenetisches Hilfsmittel. Zool. Anz. 147, 116—121.
Párducz B. 1962: Studies on reactions to stimuli in ciliates. IX. Ciliary coordination of right spiralling Paramecia. Ann. hist.-nat. Mus. hung., Pars Zool., 54, 221—230.
Sikora J. 1966: Immobilization by homologous antiserum and antigenetic tranformation in *Paramecium aurelia* in relation to the ionic composition of medium. Acta Protozool. 4.

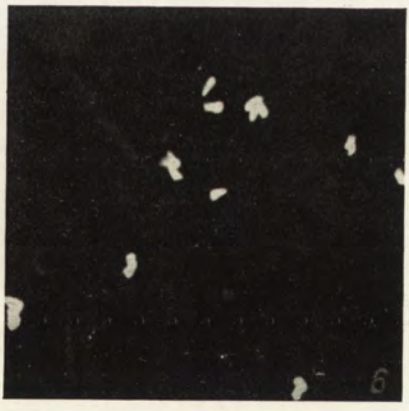
EXPLANATION OF PLATES I—III

1—6: Photomacrographic registration of paths (time of exposure 5 sec.) covered by *Paramecium aurelia*, serotype 51 A under of homologous antiserum at concentration 1:100 in phosphate buffer of Michaelis (modified) at pH 6.8. Registrations taken since the moment of adding antiserum, 1: 1 min., 2: 2 min. 45 sec., 3: 6 min., 4: 12 min., 6: 25 min. Magnification of photograms 1—6 approx. 18×
7—12: Typical motor responses of *P. aurelia* serotype 51 A under the influence of homologous antiserum. Conditions of experiment the same as in: 1—6
7,8: Movement of the FLS type with avoidings. Magnification approx. 65×
9,10: Path covered by *P. aurelia* during transition from the movement type CCR to FRS or inversely from FRS to CCR
11: Path covered by *P. aurelia* during FRS. Typical forward movement prior to immobilization. Corresponds to Pl. I 4
12: Characteristic short-lasting "vibration" of the immobilized *P. aurelia*. Corresponds to Pl. I 6. Magnification: Pl. II 9—12, approx. 85×
13: Paths covered by *P. aurelia* 51 A in buffer without serum, 1 min. after placing the ciliates on a glass plate
14: Paths covered by *P. aurela* 51 A in buffer with diluted nonhomologous serum anti-51 B, in concentration 1:100, 1 min. after placing the ciliates on a glass plate
15—18: Paths covered by *P. aurelia* 51 B, and 51 A immobilized by homologous anti-51 A serum. 15: 20 min., 16: 25 min., 17—18: 30 min. since the beginning of experiment. Magnification: Pl. III 13—16 approx. 21×, 17: approx. 42×, 18: approx. 50×









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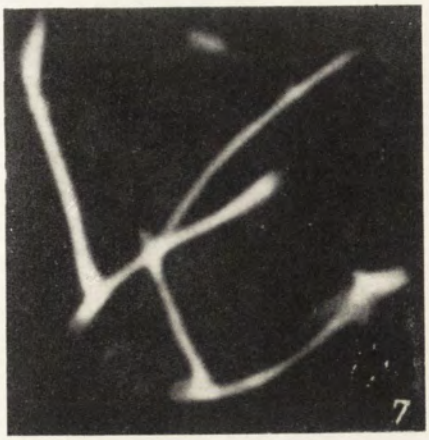

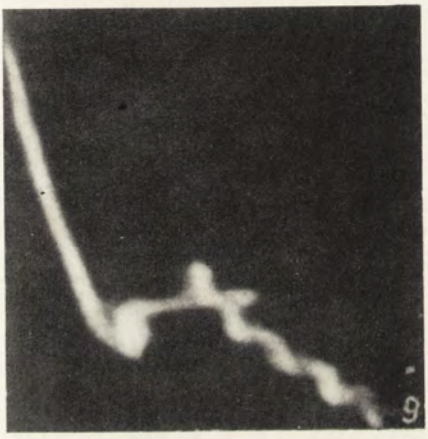

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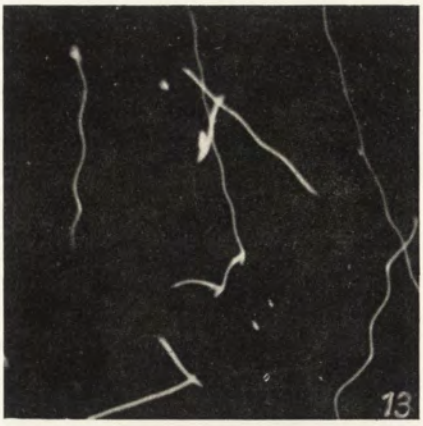

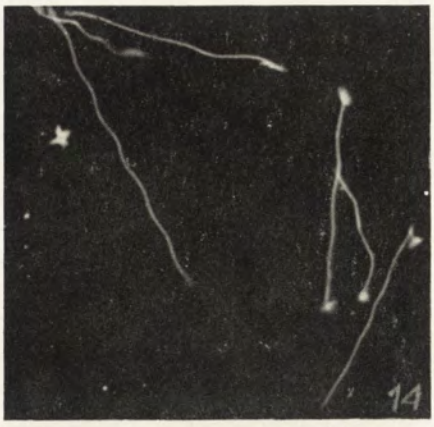

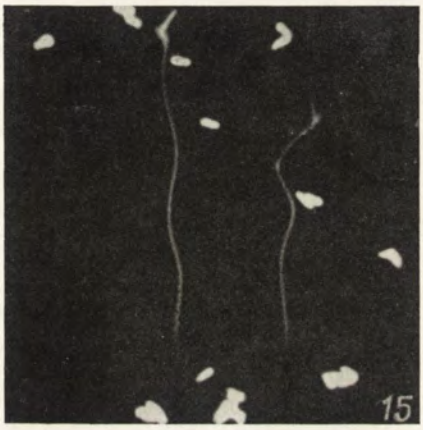

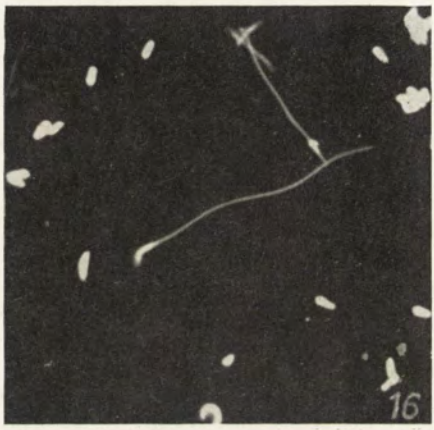

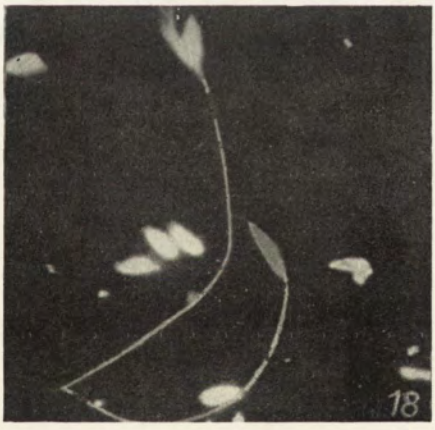

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Remarks on feeding and pathogeny of *Trichophrya (Suctoria)*

Uwagi o odżywianiu się i patogenności *Trichophrya (Suctoria)*

In the course of investigation on fishes of the Mazury Lake district, embracing the lakes Gołdapiwo, North Mamry, Kisajno, Dobskie, Dargin and Arklity, an infection with a protozoon was stated in *Coregonus albula, Perca fluviatilis* and *Esox lucius*. The protozoon parasite was first described by Prost (1952) as *Trichophrya intermedia*. The aim of the present study was to ascertain its possible pathogeny, its manner of feeding as well as the dependence of its occurrence on the character and conditions of environment. Since *Trichophrya* is a comparatively little known form, its description is also included into this study.

Observations were carried out mostly in the lake Arklity in which the study of fish parasites was performed during summer months in 1957—1963. It is a small lake of a pond character, pertaining to the Wegorzewo Fishery Establishment. *Esox lucius, Perca fluviatilis, Rutilus rutilus, Tinca tinca, Carassius carassius* and *Leucaspius delineatus* are common in it, occasionally occur *Acerina cernua, Gasterosteus aculeatus* and *Anguilla anguilla* as well. The parasite infected mostly *Perca* and — rather scarcely — *Esox*. Its pattern of distribution can hardly be accounted for by significant differences in the environment condition — as it was done by Parisi (1920) for *Alosa finta* from the Lake Como, considering the pond character of the small lake. Infection of *Perca* by *Trichophrya* increased permanently from 1957 to 1963, and the highest invasion occurred in 1962 and 1963. The summer 1963 was extremely hot. The highest degree of infection was found in the summer months, being much lower in spring and in autumn.

The parallel examination conducted by the Institute of Fresh-water Fishery showed a constant fall of *Perca* numerosity in the lake Arklity. However the mortality of fishes in the lake was not observed in a perceptible degree although the invasion of the parasite amounted in *Perca* in average over 60%, in a high percentage of specimens a mass infection was stated and symptoms of suffocation in some fishes which approached the shore, were distinct. Possibly the mortality could not be ascertained because of the special character of the lake which is overgrown with soft vegetation in which fishes may be ratained not appearing at the surface. In those which rarely were fished out, the decompcsition was so far advanced that the cause of death could not be ascertained.

Occurrence of *T. intermedia* in different fish groups and in the lakes belonging to different limnological types — from the pond-like to the mesotrophic ones — suggests that this parasite is more common than it was known till now.

Material and methods

Material was sampled mostly from *Perca fluviatilis* from the lakes Arklity and Gołdapiwo, much less from *Coregonus albula* from Dargin. Observations were carried out as well on living as on fixed material. In the first case, possibly small fragments of branchial filaments were isolated and the behaviour of the parasite was observed at low power microscope in lake water, slightly pressing the coverslip. For the histological procedure single branchial arches or their fragments or groups of arches were taken from a possibly fresh material, and fixed in the Bouin's or Zenker's fluids. Smear preparations were fixed in absolute ethanol. Sections were stained with haemalaun and eosin or with Weigert's haematoxylin. For determining the food particles in cytoplasm, the bromophenol blue method of Mazia, pyronine-methyl green of Brachet and the Feulgen's test were applied.

The degree of infection of fishes was determined on the ground of extensiveness and intensity of invasion. The parasites on gills were not counted but their number has been evaluated visually and determined as: "single", "moderate", "numerous", "mass occurrence".

I wish to express my thanks to all those who helped me in the course of the present investigations. To prof. dr. E. Grabda for giving accommodation in his laboratory for carrying out a part of the present research, to Miss. T. Einszporn for her help in selection of material for microphotography, to Mr. C. Nagieć for executing microphotographs, to doc. dr. Z. Bańkowski for performing the histochemical reactions, at last to dr. M. Kraińska for analysis of preparations, for reading the type script and for her valuable remarks.

Systematic position and morphology

Suctorians of the fish gills have been known since a long time. Bütschli (1889) — basing on the unpublished drawing of Lieberkühn — gave first the description of a species living on the gills of fresh water fishes and called it *Trichophrya piscium*. This species from the gills of *Esox, Perca* and *Acerina* in Europe was subsequently mentioned by Sand (1901) and Collin (1912). Parisi (1920) found the same species on the gills of *Alosa finta* var. *cuvier* from the lake Como in Italy and gave its description.

Subsequently three new species of *Trichophrya* from the fish gills were found by Davis (1937, 1942, 1947) in the fresh waters of North America. Davis gave them names of the species of fishes on which they were found: *Trichophrya micropteri* from *Micropterus dolomieu, T. ictaluri* from *Ictalurus punctatus* and. *T. salvelini* from *Salvelinus fontinalis*. The fifth species, similarly as the first one occurs in Europe. Prost (1952) described it as *T. intermedia* from the gills of fry of *Salmo salar* in Poland. The sixth species *T. sinensis* Chen, 1955 was described from gills of *Ctenopharyngodon idellus, Mylopharyngodon piceus, Aristichthys nobillis* and *Hypophthalmichthys molitrix* in China (Chen 1955, 1956 a, b).

Occurrence of the european species (Table 1) is not connected with a single definite fish species. Both seem to be equally distributed and have some common hosts. *T. piscium* was found in *Esox, Perca* and *Acerina* in Germany and in *Alosa finta* in Italy (P a r i s i 1920), *T. intermedia* was stated in *Salmo salar* (P r o s t 1952) in Poland. I found them in *Coregonus albula, Perca* and *Esox* in several Mazury Lakes in Poland. L o m (1960) found them also in *Silurus glanis, Salmo trutta* var. *fario* and in *Perca fluviatilis* in Czechoslovakia, at last B o g d a n o v a (1962) reported it in *Coregonus lavaretus* in the Leningrad district and L j u b a r s k a (1963) in fry of *Esox* in Kujbyšev Reservoir in USRR.

The dimensions (Table 1) and comparison of description of both species indicate that they are quite similar and the difference between *T. intermedia* and *T. piscium* consists only in the lack of the additional bundle of tentacles or additional single tentacle in *T. piscium*.

Table 1

Body dimensions (in μ) of *Trichophrya piscium* and *T. intermedia* according to different authors

	T. piscium	*T. intermedia*			
	Parisi (1920)	Prost (1952)	Lom (1960)	Bogdanova (1962)	Kozicka
Body length	100	63—120	55—111	53.3—69.7	52—116
Body width	50	30—92	41—78	24.6—53.3	33.7—80
Number of tentacles on anterior end	20—30	15—30	7—30	8—16	12—30
Number of tentacles on posterior end	none	5—11 (in drawing)	—	2—5	6—14
Length of tentacles	30	—	43 (width 1.3)	12—21	variable, may overpass half of body length
Dimensions of Ma	length 25—30	23—46×10—26.5	13—25×15—42	20.5—24.6	20—42
Dimensions of Mi	4—5	3.5—4.2	3—7	3.5—4.2	4—8.6
Host	*Esox lucius* *Perca fluviatilis* *Acerina cernua* *Alosa finta*	*Salmo salar*	*Salmo salar* *Silurus glanis* *Perca fluviatilis* *Lucioperca lucioperca* *Coregonus albula*	*Coregonus lavaretus*	*Esox lucius* *Perca fluviatilis* *Coregonus albula*

Culbertson and Hull (1962) after having examined Suctoria of the genus *Trichophrya* in 5 fish species originating from various places of North America, came to conclusion that the presence of additional bundles and additional tentacle is such a common feature in Suctoria of the genus *Trichophrya* that it cannot serve as a diagnostic character within this genus. Considering this fact, he included all the new species described by Davis (1937, 1942, 1947) as well as *T. intermedia* Prost, 1952 to the synonims of *T. piscium* Bütschli, 1889. It seems possible that the presence of an additional bundle which occurs occasionally, was overlooked in *T. piscium* by Bütschli and Parisi. In the course of the present investigation, this structure could not be found in any of the dozen of *Trichophrya* individuals form the gills of *Esox* in the Arklity Lake. It seems reasonable to postpone including *T. intermedia* to the synonims of *T. piscium* till the question would be firmly documented by the methods applied by Culbertson and Hull (1962) to the American species.

Suctorians found by me on the gills of *Esox, Perca* and *Coregonus* (Pl. I 1, 2) and identified by me as *Trichophrya intermedia* Prost, have an irregular body shape: it is ovoid or sphaerical often pear-shaped. The anterior body end is rounded, the posterior one is of variable shape depending on the place of localization. I failed to observe distinct regular processes, which are seen on the micrographs of Lom (1960), in the individuals isolated from the gill tissues of *Esox*. In sections, only the penetration of the parasite anywhere it was possible could be observed, even sometimes between the epithelial cells (Pl. II 3, 4, III 5). The body dimensions are variable and fluctuate within large limits (Table 1). On the anterior body end, a bundle of tubules is present. They diverge radially outside. Their ends are slightly dilated and rounded. Every tentacle is composed of two tubules: the interior one and another superficial. This was ascertained by Rudzińska (1962) in electron microscope. The interior tubules penetrate inside the protozoon body and terminate near the macronucleus (Pl. I 2). The number and length of those tentacles fluctuates within considerable limits. Besides the main bundle at the anterior body end, in some individuals additional tentacles are present, either dispersed on the body sides or accumulated into an additional bundle at the posterior end. The additional tentacles are usually smaller than the main ones.

Presence of one principal tubule bundle and the inclination in some individuals to produce another additional bundle is — according to Prost (1952) a diagnostic feature of *T. intermedia*.

The macronucleus is big, ovoid or elongated, micronucleus is small, rounded. Contractile vacuole is single. Reproduction occurs by internal budding. It occurs frequently. I failed to observe conjugation which was described by Lom (1960). *Trichophrya intermedia* encapsuled in a thin transparent cyst was found rather frequently (Pl. IV 7, 8).

The granulous endoplasm is sometimes filled with corpuscles of various size and shape. They sometimes are so numerous that they conceal the micronucleus. Besides those slightly yellow corpuscles, some reddish-brown irregular granules may be observed, sometimes so numerous that not only *Trichophrya* but the whole gill assume an intensely reddish colour. The narrow rim of ectoplasm is homogenous.

Pathogenous effect

The pathogenous activity of *Trichophrya* upon the fish gills seems to be doubtless. Although Parisi (1920) considered *T. piscium* as a harmless or even useful commensal, already Prost (1952) stressed the destructive action of *T. intermedia* in its covering the respiratory surface of gills and in feeding at the cost of the host tissues, without analysing those processes exactly. Lom (1960) postulates that in the case of a mass infection, the pathogenous action of the protozoon is not limited to the mechanical excitation of the epithelium but penetrates deeper inside. Davis (1937, 1947 and 1956 according to Bogdanova 1962) stressed flattening and loosening of the epithelium, the direct contact with the network of capillaries as well as destruction and necrosis of tissues under the action of *Trichophrya*. Mortality of fishes is described by Davis (1937, 1942, 1956) and Bogdanova (1962). Serious sickness in in *Ctenopharyngodon idellus* at the age 2+ was observed by Ni Da-šu (1956, 1957, according to Bogdanova 1962).

The mass occurrence of *T. intermedia* on the gills of *Perca* and *Coregonus albula* was often found by me in the lakes of the Mazurian Region. The sections of the highly infected gills allowed to follow as well the localization of the parasite as some changes evoked by it in the gill tissue.

Trichophrya settles down most abundantly on the first and second gill arch although it may also be found on the two remaining arches. On the filament they lie on the side and somewhat obliquely. Tentacles are stretched forwards, slightly upwards, directed usually towards the free space between the filaments (Pl. I 2. VII 16). Concavities arise in the gill tissue beneath the parasite (Pl. II 4, III 5). Then tentacles may lean on the anterior wall of the concavity. This was stressed by Prost (1952) and Lom (1960). In the case of a high invasion they may touch also the body of another individual which lies beneath it. Under the influence of pression and perhaps of metabolism of the protozoon, epithelium may be flattened and loosened and finally degenerated and scaled off (Pl. II 3, 4). In such places the parasite touches directly the network of gill capillaries (Pl. III 5) which was already stressed by Davis (1947). As response to the irritating action of the parasite, the fish organism tries to be separated from it. Reaction comes from the mesenchyme. Fibers of connective tissue spread out of the remainder of epithelium and embrace the parasite with a thin transparent envelope (Pl. IV 7, 8). The envelope contains — as a rule — one, rarely two protozoa. Sometimes the whole filament bends over the parasite and encloses it (Pl. III 6). A slight invasion seems not to do much harm to the host. A high invasion involves not only disturbances in respiration, but destruction of epithelium and a permanent pressure may result in destruction of some lamellae or even their groups (Pl. V 9—11). If this process extends over a larger area of gills, it may involve serious sickness or even death of the host. This was described by a number of authors (Davis, Ni Da-šu, Bogdanova).

In the case of a mass invasion, the harmful action of the parasite seems to extend on the circulation system in the organ as well. The pressure on the capillary network seems to involve slackening of blood flow in its terminal sectors. Often considerable blood congestions, overfilling of vessels and slight blood effusions are observed. Presumably the softened tissues enable the passage of blood corpuscles across the wall of capillaries.

More harmfull than the uniform mass infection seems to be the mixed infection with *T. intermedia* and *Trichodina or Trichodinella* sp. simultaneously. In this case there is no free place on the gills either of the pressure of *Trichophrya* or of the irritating influence of *Trichodina* or *Trichodinella* sp. The two last parasites creep permanently over the gills as well as over the other motionless Suctoria. In the thick layer of slime coating the whole organ, single cells, blood corpuscles, and fragments of tissues are seen (Pl. V 11).

Feeding

The pathogenic action of *Trichophrya* in the case of a mass infection seems to be doubtless. In contrast to this the problem of feeding of the parasite is not sufficiently elucidated.

Parisi (1920) thinks that *T. piscium* feeds exclusively on other protozoa which live of the fish gills as well as with the material brought by water to the tentacles. Prost (1952) suggests that *T. intermedia* feeds not only on protozoa which coexist with them on the gills of fishes but on the host tissues as well. Lom — not denying the last possibility — reported that catching protozoa by *T. intermedia* was never observed by him. Davis (1956, according to Bogdanova 1962) postulates that *Trichophrya* may suck blood of the vessels of gills. At last Culbertson and Hull (1962) found that *Trichophrya* of the fish gills may feed on other protozoa.

Not finding possibility of observing the food intake by *T. intermedia*, I tried to find another way of solution of this problem by determining the nature of the "corpuscles originating of food material". For this purpose some histochemical tests were performed on the material fixed with Zenker and Bouin fluids. The bromophenol blue method of Mazia gave an intensely positive result which proved the protein character of the corpuscles. The test of Brachet controlled by action of ribonuclease (0.5%), proved that the corpuscles are ribonucleoproteins of cytoplasm evidently connected with the protein synthesis of the cell.

Indication concerning the feeding material of the protozoon was provided by the big basophil corpuscles found in cytoplasm of some individuals. They were first observed and reproduced by Davis (1947) in *T. ictaluri* and called by this author "metaplastic corpuscles". They are visible after staining with haematoxylin which reveals their resemblance to blood corpuscles. The Feulgen reaction revealing DNA confirmed the nuclear character of the corpuscles but their origin remained still obscure. Parisi found similar corpuscles stainable with nuclear dyes in cytoplasm of *T. piscium*, and concluded that they are remnants of nuclei of protozoa co-existing with *T. piscium* on the gills of *Alosa finta*.

After application of the phase contrast to the material stained with haematoxylin and eosin, only erythrocytes and big basophil corpuscles in the protozoon cytoplasm gave the phosphorescent effect. Presence of whole unimpaired erythrocytes in the cytoplasm of the parasite provided evidence that the basophil corpuscles are ingested blood corpuscles of the host. They may be found in different stages of decomposition in the host cell. Occasionally nuclei of a different structure are found (Pl. III 6, VI 12, 13, VII 15).

Although feeding of *Trichophrya* with erythrocytes seems to be beyond any doubt, nevertheless the problem of its feeding presents some ambiguities. In the first place it is not known whether the protozoon feeds also on other gill tissues except the blood corpuscles, or on other protozoa, or on the material which is brought by water to its tentacles. The observation of living material failed to give evidence of ingesting *Trichodina* or *Trichodinella* by *Trichophrya* even then when they were very numerous and crept over the body of *Trichophrya* within the reach of its tentacles. Ingestion of *Trichophrya* by *Trichodina* — as observed by J. and Z. Raabe (1961) in *Trichodina unionis* and *Conchophtirius* — has not been observed either although the behaviour of those two ciliates suggested sometimes this possibility. Other tissues of the gill seem not to be the food of the protozoon although some nuclei different from those of erythrocyts were found in cytoplasm. *Trichophrya* lie motionless in one place; a lesion caused by ingestion of tissue at this place could hardly be compensated and would lead to catastrophy — at least a local one — of both: the parasite and the host. The permanent source of food might only be blood which has a constant access to the gill filament. Erythrocytes may leave the capillaries crossing their one-layered endothelium without impairing the wall.

The way of ingesting food has not been elucidated. It may occur by means of the tubules or by pinocytosis in the other body parts or in both ways simultaneously. In favour of the first way speaks the presence of the ingestion apparatus characteristic for the whole group. The image of tubules bent towards the erythrocyte (Pl. VII 16) — which was observed only once — suggests also the first possibility. On the contrary, dimensions of nuclei found in the parasite cytoplasm which are much bigger than the diameter of the tubules seem to speak againts it. Possibility of passing of rather big organelles along thin tubules had been reported in the litterature. Rudzińska (1962) observed passage of mitochondria along the tentacles of *Tokophyra infusionum*. Lom and Vavra (1963) found sporoplasm passing along very long and thin polar filaments in *Plistophora hyphessobryoconis*.

The possibility that other body parts of *Trichophrya* participate in food ingestion is put forward by Chen Chih-leu (1955) for *T. sinensis*. This possibility is suggested by close adhering of the surface region of the protozoon to the epithelial cells or to the endothelial walls of capillaries as well as the capability of erythrocytes to leave the vessels.

The small reddish-brown granulation in cytoplasm are determined by Davis as melanin and recently by Culbertson and Hull (1962) as unreduced melanin.

Summary

In some lakes of the Mazurian Lake Region (Poland), presence of *Trichophrya intermedia* Prost (*Suctoria*) was stated on the gills of *Preca fluviatilis*, *Esox lucius* and *Coregonus albula*. The species was described. Pathogeny of the mass invasion of the parasite was stated on the living material as well as on the fixed one. An attempt was made to determine the nature of the bodies of the food origin in the cytoplasm of protozoon by means of histochemical methods. Detection of non-phagocytized erythrocytes allowed to put forward the postulation that *Trichophrya* feed on the erythrocytes of the host.

STRESZCZENIE

Stwierdzono w szeregu jezior mazurskich zarażenie skrzeli *Perca fluviatilis*, *Esox lucius* i *Coregonus albula* przez *Trichophrya intermedia* Prost (*Suctoria*). Dokonano opisu pasożyta oraz zebrano szereg spostrzeżeń dotyczących patogeniczności, odżywiania a także zależności występowania pierwotniaków od warunków i charakteru środowiska.

Masowe zarażenia przez *Trichophrya* są niewątpliwie dla ryb patogeniczne. Pasożyt osiedlając się na listewkach skrzelowych powoduje spłaszczenie, bądź zniszczenie komórek nabłonka, przylegając bezpośrednio do naczyń włoskowatych. Ucisk i ogałacanie z nabłonka, zaburzenia w procesie oddychania i krążenia doprowadzić mogą do zniszczenia pojedyńczych listewek skrzelowych czy całych ich grup. Objęcie przez ten proces większej powierzchni skrzeli może pociągnąć za sobą poważne schorzenia lub nawet śnięcia, opisane przez Davisa, Ni Da-šu i Bogdanową.

Problem odżywiania się starano się wyjaśnić drogą określenia natury "ciałek pochodzenia pokarmowego" w cytoplazmie pierwotniaka. Ogromna ich większość należy do ribonukleoproteidów. Ustalenie charakteru jądrowego dużych ziarnistości i wykrycie w cytoplazmie nie naruszonych jeszcze czerwonych ciałek krwi wykazało, że pasożyt może się żywić krwinkami. Jaką drogą odbywa się ich wchłanianie i czy stanowią wyłączny pokarm pierwotniaka, nie wykryto.

REFERENCES

Bogdanova E. A. 1962: Sosuščie infuzorii roda *Trichophrya* kak vozbuditeli massovyh zabolevanij ryb. Naučno-Techn. Bjul. GosNIORH 16, 64—67.

Bütschli O. 1888—1889: Protozoa Vol. I. In: Bronn's — Klassen und Ordnungen des Tierreichs. C. F. Winterscher Verlag, Leipzig.

Collin B. 1912: Étude monographique sur les acinétiens. II. Morphologie, physiologie, systématique. Arch. Zool. exp. gen. 51, 1—457.

Chen Chih-leu. 1955: The protozoan parasites from four species of Chinese pond fishes: *Ctenopharyngodon idellus*, *Mylopharyngodon aethiops*, *Aristichthys nobillis* and *Hypophthalamichthys molitrix*.
I. The protozoan parasites of *Ctenopharyngodon idellus*. Acta hydrobiol. sinica 2, 123—164.

Chen Chih-leu. 1956 a: The protozoan parasites from four species of Chinese pond fishes: *Ctenopharyngodon idellus*, *Mylopharyngodon piceus*, *Aristichthys nobillis* and *Hypophthalmichthys molitrix*.
II. The protozoan parasites of *Mylopharyngodon piceus*. Acta hydrobiol. sinica 1, 19—42.

Chen Chih-leu. 1956 b: The protozoan parasites from four species of Chinese pond fishes: *Ctenopharyngodon idellus*, *Mylopharyngodon piceus*, *Aristichthys nobillis* and *Hypophthalmichthys molitrix*.
III. The protozoan parasites of *Aristichthys nobillis* and *Hypophthalmichthys molitrix*. Acta hydrobiol. sinica 2, 279—298.

Culbertson J. R. and Hull R. W. 1962: Species identification in *Trichophrya* (*Suctorida*) and the occurence of melanin in some members of the genus. J. Protozool. 9, 455—459.

Davis H. S. 1937: A gill disease of the smallmouth black bass. Progr. Fish Cult. 27, 7—11.

Davis H. S. 1942: A suctorian parasite of the smallmouth black bass with remarks of other suctorian parasites of fishes. Trans. Amer. mirc. Soc. 61, 309—327.

Davis H. S. 1947: Studies of the protozoan parasites of fresh water fishes. Fish. Bull. U. S. 51, 1—29.

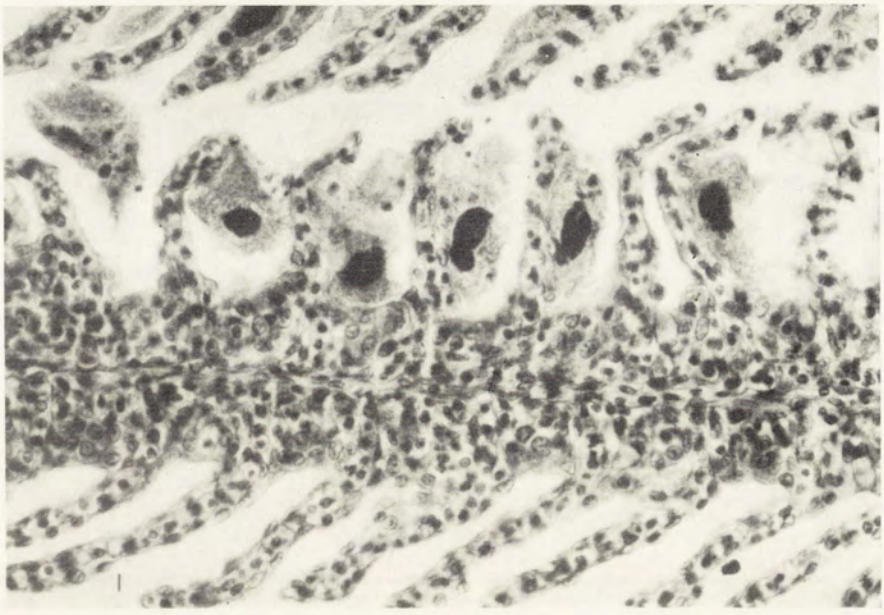
Davis H. S. 1956: Culture and disease of game fishes 2. Californica Press.

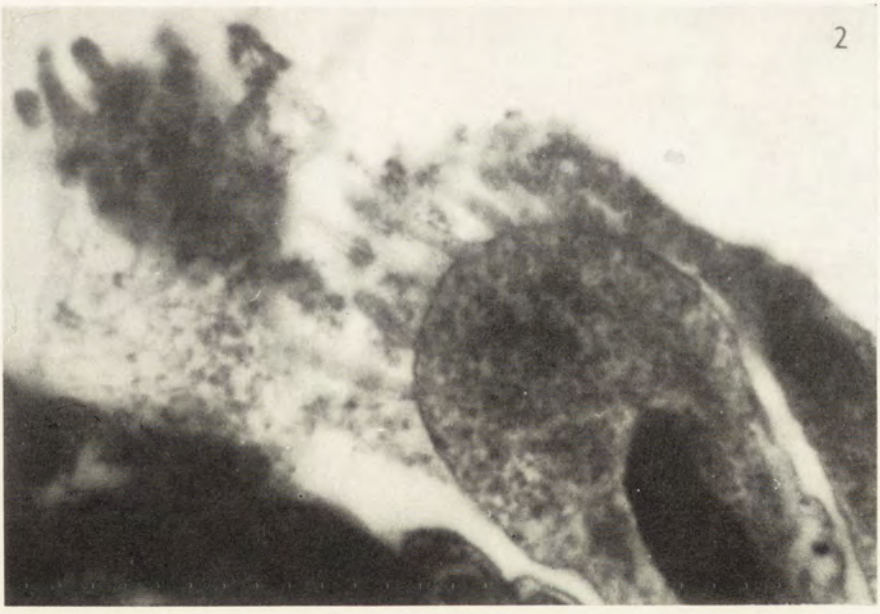
Ljubarskaja O. D. 1963: Dinamika parazitofauny molodi ščuki Volžskogo otroga Kujbyševskogo vodohranilišča. Sb. aspirantsk. rabot Kazansk. univ., estestv. nauki 54—61.
Lom J. 1961: Příspěvek k poznání parasitickych prvoku našich ryb I. *Myxosporida, Suctoria.* Zool. listy 10, 45—58.
Lom J. and Vávra J. 1963: The mode of sporoplasm extrusion in microsporidian spores. Acta Protozool. 1, 81—89.
Ni Da-šu. 1956: Sposoby presupreždenija i lečenija neskolkih osnovnyh boleznej ryb. Rybn. prom. za rubežom 4.
Ni Da-šu. 1957: Metody vyraščivanija belogo amura, černogo amura, tolstolobika i pestrogo tolstolobika. Doklad na II Plenum Komiss. po rybohoz. issl. zapadn. časti Tihogo Okeana.
Parisi B. 1920: Sulla *Trichophrya piscium* Bütschli. Atti Soc. ital. Sci. nat., Milano, 59, 63—69.
Prost M. 1952: Badania nad pierwotniakami pasożytnymi skrzeli ryb 1. *Trichophrya intermedia* sp. n. na skrzelach narybku łososia. Ann. Univ. Curie-Skłodowska, Sect. C. 6, 379—386.
Raabe J. and Raabe Z. 1961: *Urceolariidae* from fresh-water and terrestrial molluscs in Poland. Acta Parasit. polon., 9, 141—152.
Rudzińska M. 1962: The fine structure of the feeding apparatus in *Tokophrya infusionum.* 5th Int. Congr. electron Microsk. Acad. Press New York UU-12.
Sand R. 1901: Ètude monographique sur le groupe des infusoires tentaculifères. Ann. Soc. belge Micr. 24—26, 1—434.

EXPLANATION OF PLATES I–VII

Trichophrya intermedia Prost, 1952

1: *Trichophrya* on the gill filaments of *Perca fluviatilis*. 40 × 2.5
2: Single individual of *Trichophrya* with expanded tentacles; in cytoplasm the outlines of inner tentacle tubules are seen. Phase contrast, 90 × 4
3: Changes in the places of attachment of the parasite. Epithelial cells are flattened between the parasite and the filament. Phase contrast, 90 × 4
4: No epithelial cells between the parasite and the filament; concavity is seen in the gill tissue. Phase contrast, 90 × 4
5: A considerable loss of the gill tissue. Parasite adheres directly to the wall of capillary. "Metaplastic bodies" are absent in cytoplasm. Phase contrast, 90 × 4
6: Parasite is surrounded by the gill filament. In the parasite cytoplasm, two ingested cells are seen, one is unimpaired, only the nucleus remains of the other. 90 × 4
7—8: Parasite in a cyst. 90 × 4; 8 — phase contrast
9—11: Gill filaments destroyed 90 × 4
12: Parasite, in cytoplasm ingested cells are seen. Phase contrast 90 × 6.3
13: Parsite, in cytoplasm ingested erythrocytes are seen. 90×4
14: Parasite, in cytoplasm "metaplastic bodies" are seen. Phase contrast, 90 × 4
15: Parasite, in cytoplasm nuclei of ingested cells are seen. Phase contrast, 90 ×4
16: *Trichophrya*, among tentacles erythrocytes are seen. Phase contrast, 90 ×4





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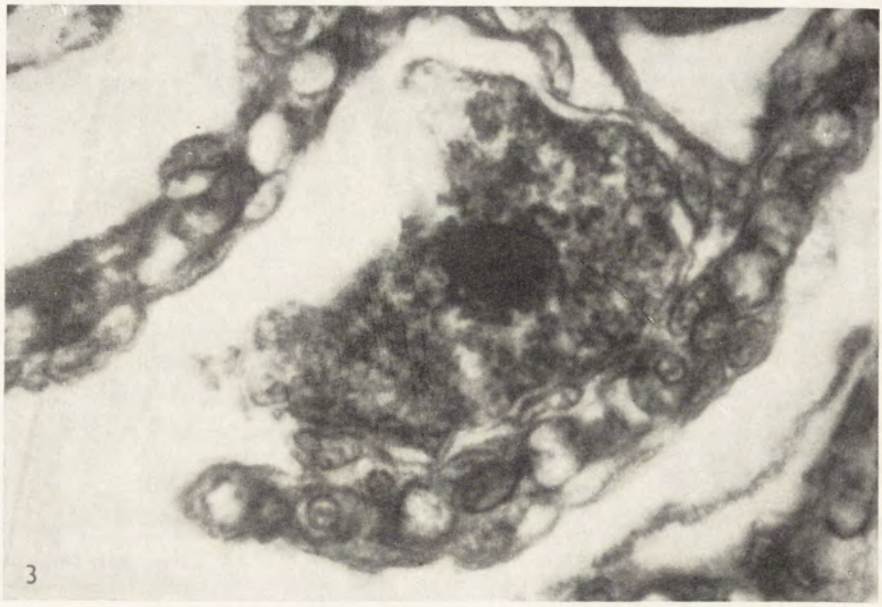

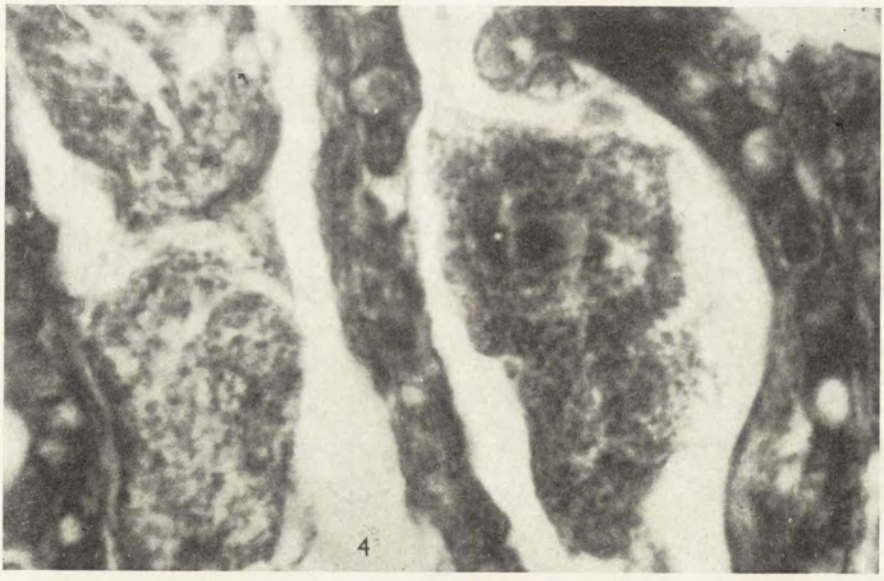

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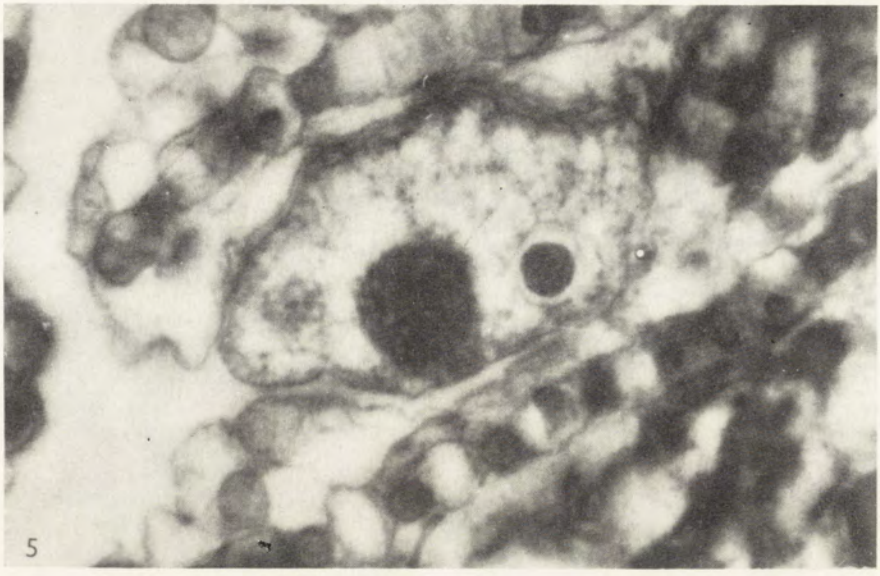

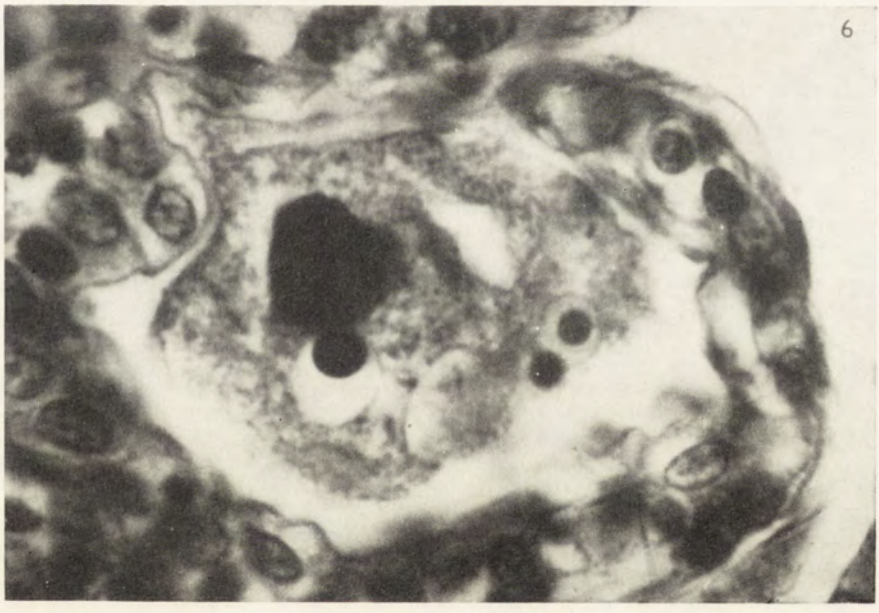

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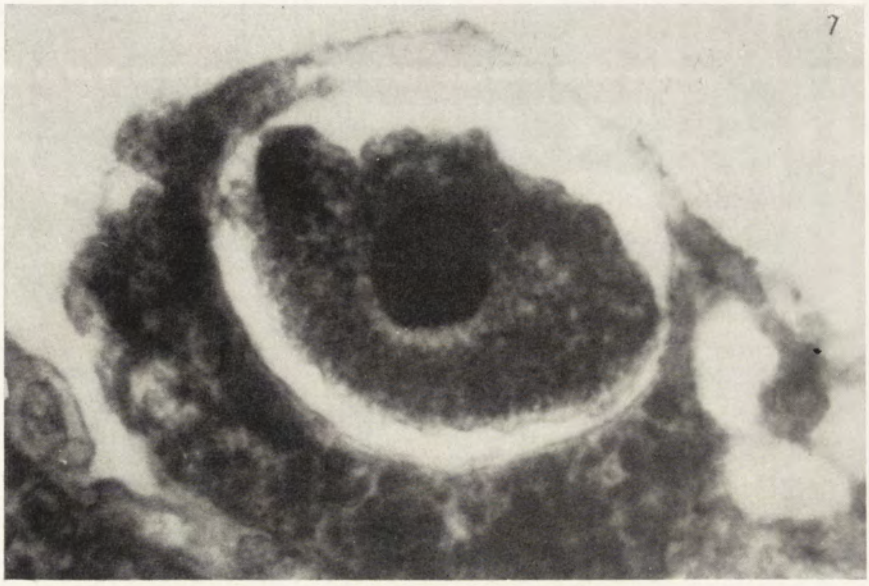

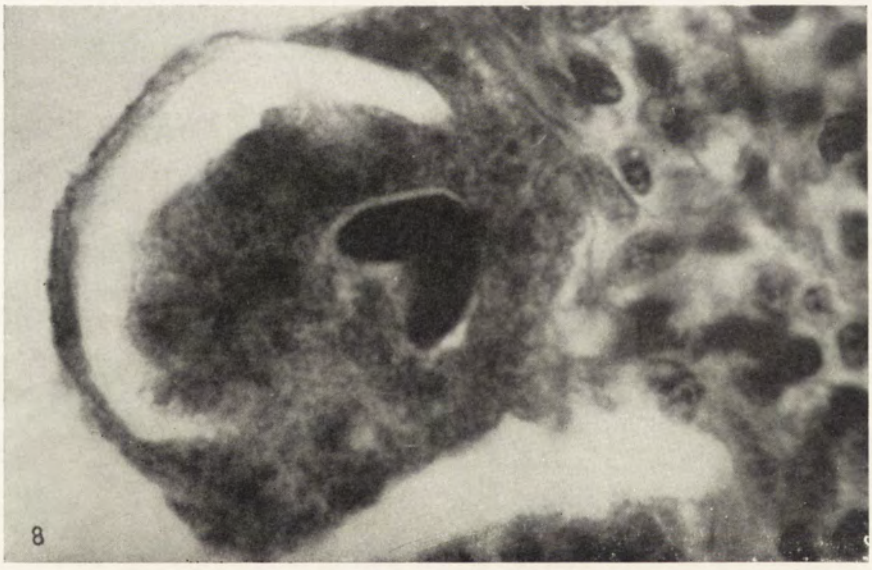

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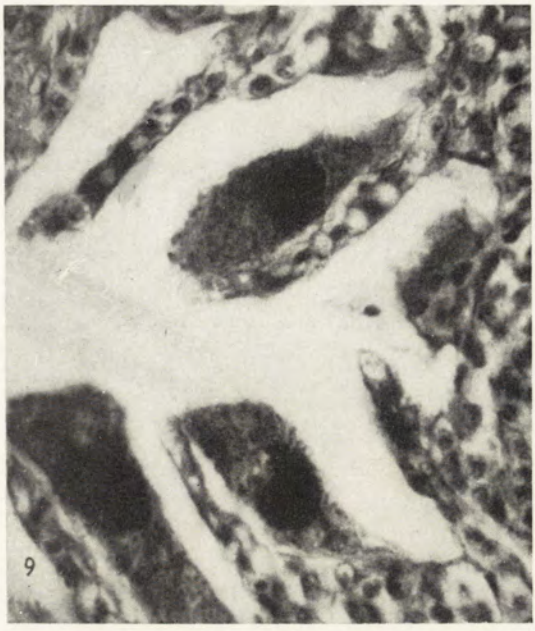

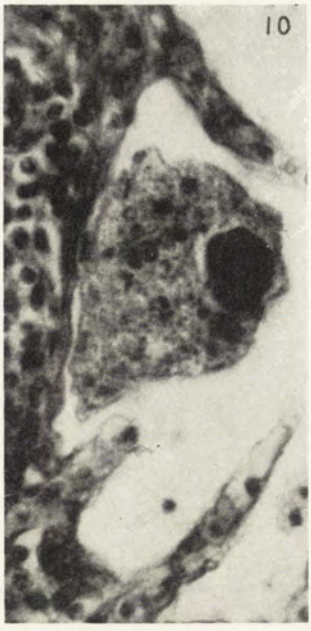

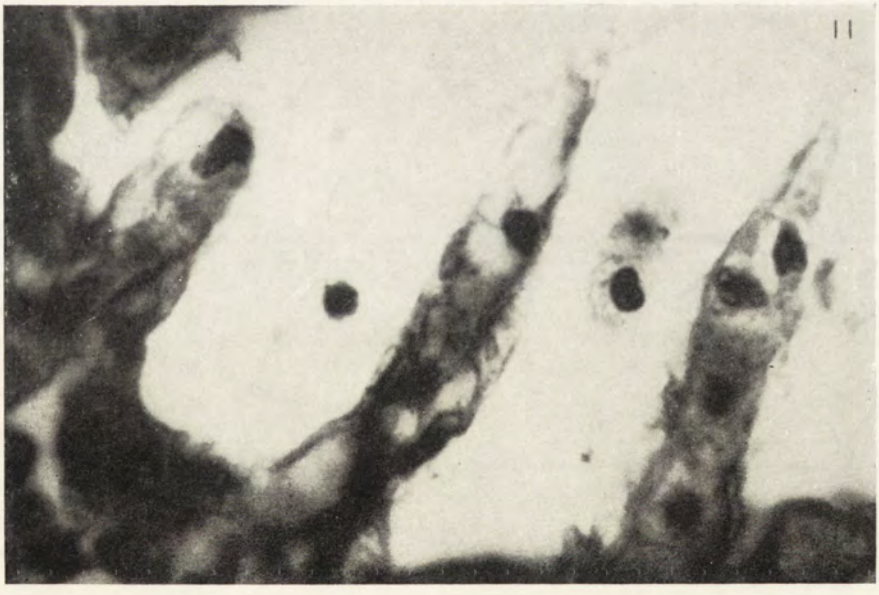

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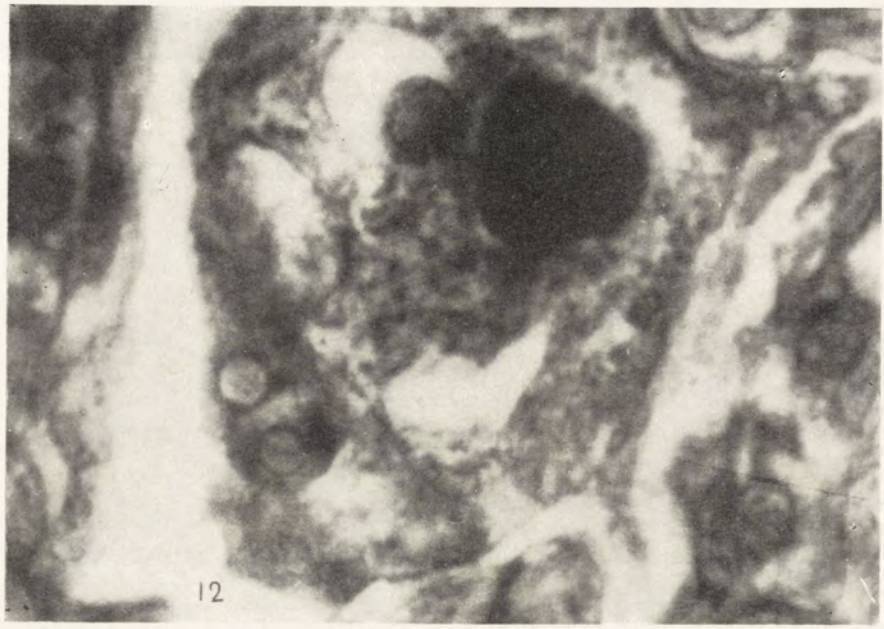

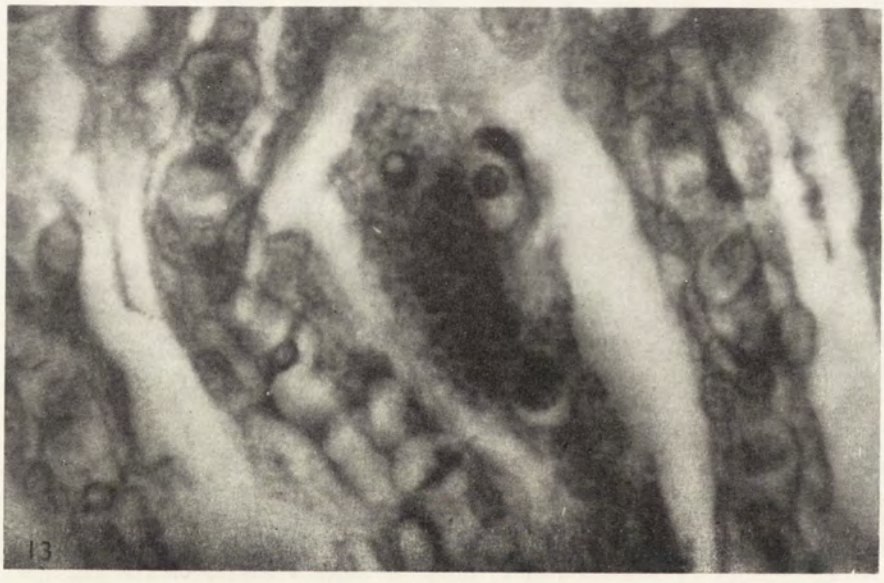

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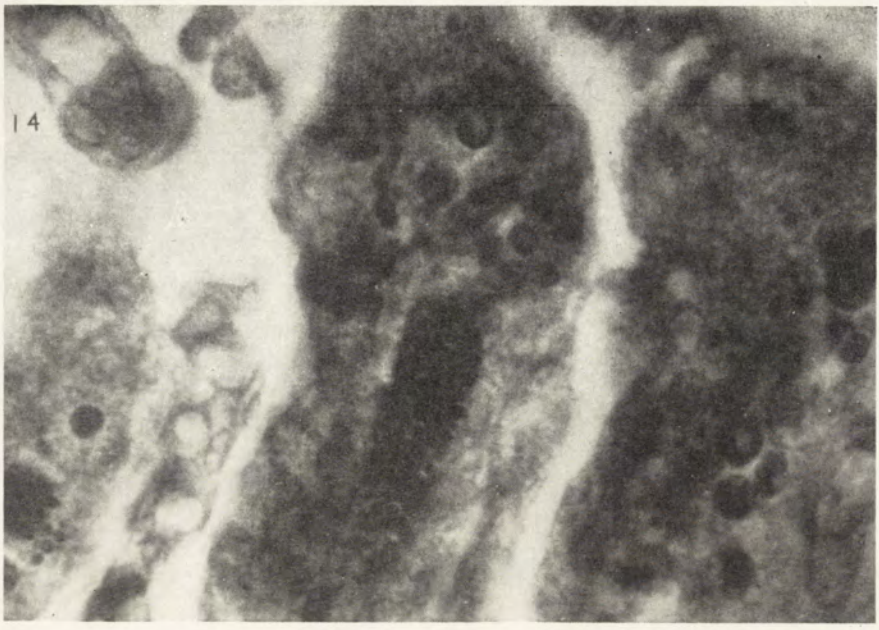

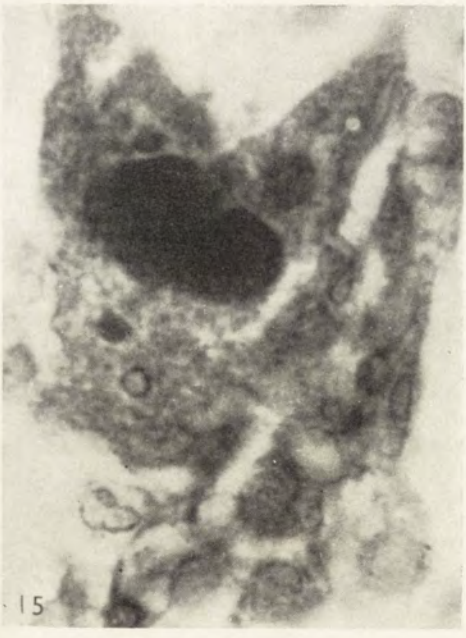

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