POLISH ACADEMY OF SCIENCES
NENCKI INSTITUTE OF EXPERIMENTAL BIOLOGY

PROGRESS IN PROTOZOOLOGY

Proceedings of
VI INTERNATIONAL CONGRESS OF PROTOZOOLOGY
Warszawa, Poland, 5-11 July, 1981

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Part I

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PART I

Editors:

S. DRYL, S. L. KAZUBSKI, L. KUŹNICKI and J. PŁOSZAJ

SIXTH INTERNATIONAL CONGRESS OF PROTOZOOLOGY

5–11 JULY 1981, WARSZAWA, POLAND

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INTRODUCTION

The Sixth International Congress of Protozoology was held at the Fryderyk Chopin Academy of Music in Warsaw between July 5 to 11, 1981. Four hundred participants from 34 countries were representing various aspects of protozoology with special attention focused on: systematics and phylogeny, genetics and morphogenesis, development and life cycles, physiology, biochemistry, immunology, motility and behaviour, ecology of free-living and parasitic protozoa. The scientific programme consisted of six invited lectures, seven symposia (including one Round Table Discussion Meeting). All lectures and the Round Table Discussion were held at plenary sessions in the Concert Hall, while symposia were run parallel as three separate meetings. In addition a number of Contributed Paper Sessions and Poster Sessions were organized as scientific meetings related to subjects of symposia.

Abstracts of presented papers were published in the pre-Congress volume[1] which was supplied to all Congress participants.

The editors decided to publish the Proceedings of the Congress as two separate, special issues of Acta Protozoologica. The first volume (part I) includes all invited lectures and material of two Contributed Paper Session in Memory of Prof. T. L. Jahn ("Motility and Behaviour") and in Memory of Prof. T. M. Sonneborn ("Genetics and Morphogenesis"), and materials of Symposium "Ultrastructural and Molecular Background of Motility".

The second volume (part II) will include material of symposia and Round Table Discussion on Phylogenetic Relationships among *Protozoa*.

The organizers of the Congress gratefully acknowledge the financial support from United Nations Environment Programme (Kenya, Nairobi), from International Union of Biological Sciences (France, Paris) and from the Polish Academy of Sciences.

At the Opening Plenary Session hold during afternoon, July 5, 1981 the Congress was addressed by:

Dr. Stanisław DRYL, President of the Congress;

Dr. Aleksander GIEYSZTOR, President of the Polish Academy of Sciences;

Dr. Barclay McGHEE, President of the Society of Protozoologists;

Dr. Adam URBANEK, Secretary of the Biological Division of the Polish Academy of Sciences;

Dr. John J. LEE, Secretary of the International Commission of Protozoology.

It should be pointed out that the Fryderyk Chopin Academy of Music in Warsaw provided exceptionally good facilities and this without doubt contributed in a good way to friendly atmosphere throughout the Congress, both in scientific and cultural aspects.

After vivid discussions the International Commission of Protozoology has decided to organize the next Seventh Congress of Protozoology during summer 1985 in Nairobi, Kenya. A non-official invitation for the Congress was presented by Dr. Mutuku Mutinga (The International Centre of Insect Physiology and Ecology, Nairobi Kenya) was unanimously accepted by Congress participants at the Closing Session on July 11, 1981.

Stanisław Dryl
President, VI International
Congress of Protozoology

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[1] Progress in Protozoology, Abstracts of papers submitted to VI International Congress of Protozoology, Warszawa, Poland, July 5–11, 1981, pp. 428.

SCIENTIFIC PROGRAMME

Sunday, July 5

OPENING SESSION

Plenary Lectures

William TRAGER — Recent Advances in the *in vitro* Cultivation of Parasitic *Protozoa* and their Significance for the Control of these Parasites

Jurij POLJANSKY — Intraspecific Variations and Species Concept in *Protozoa*

Leszek KUŹNICKI — Protozoology in Poland: Past and Present

Monday, July 6

Symposium C	Symposium F	Symposium B
Cellular Membranes: Structure and Function	Mutualistic (Symbiotic) Relationship	*In vitro* Cultivation of Parasitic *Protozoa*
Poster Sessions: Part B, Part C, Part F and Part III, Part IV, Part V, Part VI		
Meeting of International Commission of Protozoology		

Tuesday, July 7

CPS of Symposium C	Symposium F – continued	CPS of Symposium B
CPS – Section VI: Ecology of Free-Living *Protozoa*	CPS – Section V: Motility and Behaviour in Memoriam of Professor T. L. Jahn	CPS – Section III: Antigenic Analysis and Immunogenecity
	Film session	
CPS – Section VI: Ecology of Free-Living *Protozoa* – continued	CPS – Section IV: Action of External Agents and Drugs	CPS – Section – III – continued Meeting of the Associated Protozoological Societies

Wednesday, July 8

Poster Sessions: Part A, Part D, Part E and Part I, Part II, Part VII, Part VIII

Thursday, July 9

Plenary Lectures

Peter J. BRUNS	Genetic Engineering in *Tetrahymena*
John B. TUCKER	Assembly and Patterning of Microtubular Structures
Yoshinori NOZAWA	Environmental Adaptation of *Tetrahymena* Membranes: Role of Membrane Lipids

Symposium D	Symposium E	Symposium A and Contributed Paper Session —
Cytoplasmic Organelles (e.g., Mitochondria, Kinetoplasts, Hydrogenosome, Glycosome)	Ultrastructural and Molecular Background of Motility Meeting of the International Commission of *Protozoa*	Section I: Variation, Life Cycle, Systematics and Phylogeny of *Protozoa*

Friday, July 10

Contributed Paper Session	Contributed Paper Session	Contributed Paper Session
Section VIII:	Section II:	Section VII:
Cytoplasmic Organelles and Metabolism	Genetics and Morphogenesis in Memoriam of Professor Tracy M. Sonneborn	Ecology of Parasitic *Protozoa* and Host-Parasite Relationships

The Protozoological Society — Meeting and Banquet at the Europejski Hotel

Saturday, July 11

Round-Table Discussion on Phylogenetic Relationships Among *Protozoa*

Closing Plenary Session

REMARKS OF THE CHAIRMAN OF SCIENTIFIC SESSIONS

The Sixth International Congress of Protozoology which during seven days dealt with symposia, lectures, sessions and social events came to its end. In this short concluding address it is not possible to evaluate each symposium, session and lecture. However, I hope to express the opinion of the majority of participants that our Congress has succeeded in achieving its aims, and we may be generally satisfied with its achievements.

The main source of the general success of all scientific sessions were chairpersons and speakers. Therefore, allow me in the first place to thank the chairperson and speakers for their hard work. I also want to express sincere thanks to all conveners and those who are working with projectors and did organize the poster sessions. They helped to organize the sessions and ensured the smooth work of the whole Congress.

One of the aims of the scientific congress is formulating and summarizing the most substantial ideas expressed in lectures, contributed papers, posters and discussions about main topics of plenary sessions, symposia and sessions.

This implies an obligation for invited speakers of the plenary lectures as well as for the chairpersons of symposia to prepare the text for the Post-Congress volume. The completed manuscript of summary reports and plenary lectures should be prepared by the chairpersons during two and a half months after the Congress and sent to the Secretary General, Dr. S. L. Kazubski not later than October 1, 1981.

The chairpersons of the Contributed Paper Session are also kindly asked for the summary reports if it is possible for them to summarize the most substantial ideas of the session in a short logical form.

We expect that the Post-Congress volume will be printed till 1983. The copies of Guidelines for Authors of Plenary Lectures and Summary Reports are to be taken from the table just outside the Concert Hall.

The scientific programme of the Sixth International Congress of Protozoology in comparison to the previous congresses — was in my opinion, more compact but still it must be improved in the future.

I also hope that one of the results of this Congress may be to introduce the participants to pay a further visit to our country, where they always be welcome.

I wish you all a very pleasant time during the rest of your stay in Poland, and the best of success in your further scientific work. Thank you and good luck to all of you.

July 11, 1981

Leszek Kuźnicki

The participants of the Congress in the park behind the Fryderyk Chopin Academy of Music. July 7th 1981

PROGRESS IN PROTOZOOLOGY
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part I, pp. 7–21, 1982

Invited Lecture

The Cultivation of Parasitic *Protozoa*

William TRAGER

The Rockefeller University, 1230 York Avenue, New York 10021, USA

The cultivation of parasitic protozoa and helminths has been reviewed recently (Maramorosch and Hirumi (ed.) 1979, Rowe and Hirumi (ed.) 1980, Taylor and Baker 1978, Trager 1980). Here I will confine my discussion to recent work on parasitic protozoa with special reference to the uses to which the cultures are being put. I consider cultivation not as an end in itself but rather as a means to many other ends. In the simplest case, it permits the organism to be brought into laboratories where it otherwise could not be kept. For example, before the cultivation of *Plasmodium falciparum*, the only sources of this parasite were human infections, infections in splenectomized chimpanzees or, since 1965, infections in certain small New World monkeys, particularly *Aotus trivirgatus*. *Aotus* monkeys are now in short supply and very expensive. But now that we can grow the parasite continuously in human erythrocytes *in vitro* (Trager 1980), laboratories of molecular and cell biology that would not otherwise have studied *P. falciparum* are contributing to our understanding of this organism. Cultivation normally permits the production of larger amounts of parasite material than is otherwise obtainable. This is useful for some kinds of biochemical work and for immunological and serological studies. In perhaps its most important aspect, cultivation places the organism under more nearly controlled conditions. This control is greatly increased if the cultures are axenic and still more so if the organism can be grown in a defined medium. It is worth noting that in the groups, *Apicomplexa* and the *Microspora*, not one

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Paper presented on Plenary session on July 5 at VI International Congress of Protozoology, Warszawa, Poland, 5–11 July 1981.

species has been grown axenically, that is, apart from its living host cell. And as yet, relatively few species of parasitic protozoa have been grown in a defined medium, an essential condition for determining their nutritional requirements.

It is in relation to these uses of the cultures that I would like to discuss the work on cultivation of parasitic protozoa. I cannot think of a better group of organisms with which to begin than the trypanosomatid flagellates. Here we find many different positive accomplishments and yet much scope for further work. The entire complex life cycles of trypanosomes have now been reproduced *in vitro*. It was less than five years ago that Hirumi (Rowe and Hirumi 1980) first obtained growth of the bloodstream forms of *Trypanosoma brucei in vitro* by using an appropriate culture medium combined with a suitable feeder layer of tissue culture cells. A modification of Hirumi's method has been used to grow excellent cultures of *T. rhodesiense* bloodstream forms (Hill et al. 1978), but no continuous cultures at 37° of the bloodstream form of *T. congolense* have yet been reported, though such forms have been maintained up to 21 days in dermal explants (Gray et al. 1979). By manipulating temperature and feeding of the cultures of *T. brucei*, it was possible to produce procyclic forms, epimastigotes and metacyclic forms at 25° and again get bloodstream trypomastigotes when the temperature was raised to 37°. The precise conditions determining this cycle of development (Rowe and Hirumi 1980) have not been identified. Lowering the temperature to 25° was essential to initiate it. The cultures were held without change of medium and without opening the flasks and many trypanosomes died so that their products of disintegration might have had an influence. In any case, midgut and proventricular forms appeared and the cultures became non-infective to mice. When medium changes were again begun with the cultures still at 25°, small numbers of epimastigotes and finally of metacyclic forms appeared, and the cultures were again infective to mice. After 40 days of such low temperature growth, 26 out of 30 cultures had reacquired infectivity. If such cultures were again incubated at 37°, typical infective bloodstream trypomastigotes reappeared and multiplied rapidly. These results have proved difficult to reproduce with other strains.

However, tsetse fly tissues, in particular, head and salivary glands, will support *in vitro* a more complete cyclic development from procyclic culture forms to epimastigotes to metacyclic trypanosomes which are infective to mice (Cunningham and Taylor 1979). Cultures prepared with 25 or fewer head-salivary gland explants rarely became infective, but those with 27 to 50 such explants consistently produced infective trypanosomes. Antigenic analysis of these metacyclic trypanosomes by immunofluorescence shows that they correspond to those produced in tsetse flies (Gardiner et al. 1980). Similar results

have now been obtained with *T. congolense* (Gray et al. 1981) by initiating cultures with trypanosomes from the mouthparts of infected *Glossina mortisans* placed with bovine dermal collagen explant at 28°. Primary cultures became infective for mice after 2 weeks and remained infective continuously up to 76 days. Subcultures made into medium with proboscides from uninfected flies remained infective up to 215 days. The cultures produced as many as 1.6×10^6 infective organisms every 2 days and all the morphological forms normally seen in the proboscis of infected flies were present. It is clear that we may be approaching the time when it will be possible to define and manipulate the conditions controlling the differentiations that characterize the complex life cycles of the African trypanosomes.

Cultures of the bloodstream forms of *T. brucei* are meanwhile already in use for study of the remarkable phenomenon of antigenic variation. This variation occurs *in vitro* much as it does *in vivo*, thus finally ruling out any role of the immune system of the host in the induction of antigenic variation (Doyle et al. 1980). Tissue culture systems very like that of Hirumi for *T. brucei* have also been used in the cultivation of bloodstream forms at 37° of the rather host-specific trypanosomes of rodents, *T. lewisi*, *T. acomys*, and *T. musculi* (Albright and Albright 1978, El On and Greenblatt 1977, Viens et al. 1977).

Among the stercorarian trypanosomes of mammals, *T. theileri* has repeatedly been observed to grow in tissue culture at 37° with formation of trypomastigotes (McHolland-Raymond et al. 1978). In general with such trypanosomes, cultures grown at 26° reproduce the invertebrate cycle including the formation of infective forms. This is true also of trypanosomes of birds (as *Trypanosoma avium* — Baker 1966) and of trypanosomes of fish and other cold-blooded vertebrates (Quadri 1962). With the latter, the interesting problem remains of producing *in vitro* the large striated blood forms. With many of the stercorarian trypanosomes, bloodstream forms have been obtained only in the presence of a feeder layer of tissue culture cells, as is true with the salivarian forms. At least one strain of *T. theileri*, however, has been grown continuously at 37° in a semi-defined medium (Sollard and Soulsby 1968).

T. cruzi, medically the most important of the stercorarian trypanosomes, will readily produce its vertebrate stages, intracellular amastigotes maturing to trypomastigotes, in a variety of tissue cultures. This has been known since the early work of Meyer and Xavier de Oliveira (1948) and Neva et al. (1961). More recently, however, all the stages of the life cycle of *T. cruzi* have been obtained in non-living media, that is, axenically, at 37° (Pan 1971, 1978).

Whereas the amastigotes of *T. cruzi*, its intracellular multiplicative

form, will develop in non-living media, the superficially similar amastigotes of *Leishmania* have never yet been grown extracellularly. It is interesting to note that, despite superficial similarities of the amastigotes of *T. cruzi* and *Leishmania*, their physiology may be very different. Amastigotes of *T. cruzi* develop directly in the cytoplasm of host cells such as muscle or fibroblasts, whereas those of *Leishmania* develop in the lysosomal vacuole of macrophages. While many investigators have obtained brief development of various species of *Leishmania* within macrophages *in vitro*, only Lamy and others using his dog histiocytoma line have obtained continuous cultivation of *L. donovani* amastigotes (Lamy 1972). More recently, Chang (1980) has developed a still better system for continuous *in vitro* production of amastigotes of *L. mexicana amazonensis* using the mouse macrophage line J744EB.

In sharp contrast to the difficulty of *in vitro* growth of amastigotes of *Leishmania* is the apparent ease with which the promastigotes of many species can be grown. Here recent progress is concerned with growth in defined media and this will be considered later. There is another aspect, however, of the growth of promastigotes of *Leishmania* to which too little attention has been given, namely, their infectivity. Many cultures of promastigotes are said to lose infectivity completely, whereas others are more or less infective. There are indications that the previous conditions of culture affect infectivity (Giannini 1974), but this is only now being investigated in more detail (J. Keithly, personal communication). It is possible that promastigotes in sandflies may undergo developmental changes culminating in high infectivity, and that we do not yet know how to produce such forms *in vitro*. Several insect cell culture media have proved useful for culture of leishmanial promastigotes (Rowe and Hirumi (ed.) 1980).

The fact that some differentiated stages of the life cycle do not occur under ordinary *in vitro* conditions points to the value of cultures in studies on the developmental cycle. By manipulating the culture conditions, one can try to find out what conditions are responsible for the differentiated development. A simple illustration is provided by the encystation of *Entameba invadens*. Axenic cultures of this amoeba can be made to encyst by placing them in an appropriate medium (Rengpian and Bailey 1975). On the other hand, encystation (Cleveland and Sanders 1930, Diamond 1980) of axenically grown *E. histolytica* has never been obtained, despite many efforts (Diamond 1980). The formation of round cyst-like bodies with a thick outer wall was observed when axenic *E. histolytica* were fed starch and exposed to bacterial endotoxin, and were then placed in a minimal medium with epinephrine and theophylline at 33° (Mitra and Murti 1978). When placed in fresh growth medium, these bodies hatched to yield

trophic forms. Of the several species of the intestinal flagellate *Giardia* that have been grown axenically (Meyer 1970, 1976), none has been seen to encyst in such cultures. Excystation, however, occurs readily if the cysts are first exposed to a synthetic gastric juice of pH 1.3–2.7 and then placed in axenic culture medium (Bingham and Meyer 1979).

Parasites in culture may lose infectivity or pathogenicity for the host. If infectivity depends on differentiation of special infective forms, as the metacyclic forms of trypanosomes, and if these forms are not produced in the cultures, the lack of infectivity is readily explained. But in other instances no ready explanation is available. For example, *E. histolytica* when grown axenically loses its pathogenicity for experimental animals (Das et al. 1979) and this is only slowly restored when the amoebae are grown with bacterial associates. While this seemed rather mysterious, some of the mystery has been removed by the recent finding that pathogenicity could be restored to cultures grown axenically for 5–6 years by supplementing the medium with cholesterol (Meerovitch and Ghadirian 1978). The number of passages needed in cholesterol-supplemented medium to restore a certain degree of pathogenicity for hamsters was proportional to total period in culture, being much larger for a line isolated in 1924 than for one isolated in 1967. Once restored, pathogenicity persisted for a long time after cholesterol treatment was stopped.

Of special interest with regard to loss of infectivity and pathogenicity in culture have been the studies of Honigberg with several species of trichomonads (Honigberg 1979). A strain of *Trichomonas gallinae* highly pathogenic for pigeons lost pathogenicity after 17–21 weeks of axenic culture, and some strains even lost their infectivity. If the rate of multiplication in culture was slowed by omitting carbohydrate from the medium, the rate of loss of pathogenicity was also slowed, suggesting the gradual dilution of a cytoplasmic factor. In keeping with this was the finding that an attenuated strain exposed to both DNA and RNA from a virulent strain regained some pathogenicity, as measured by a mouse assay, and also regained infectivity for pigeons. However, when cultures of *T. gallinae* were grown for over a year in presence of chick liver cell cultures, there was no loss in virulence, despite a high growth rate. Evidently, under these conditions the presumed cytoplasmic factors are not diluted away. It would be interesting to know precisely the factors derived from the tissue culture responsible for this effect. The results with cholesterol and *Entamoeba* suggest some approaches to this problem. Results similar to those with *T. gallinae* have been obtained with *T. vaginalis*, the human parasite. Prolonged axenic cultivation results in loss of pathogenicity for mice.

The ultimate goal of culture work is to develop a defined medium, or a series of defined media, in which the parasite will develop axenically through its entire life cycle and will retain infectivity. This goal has been achieved for some of the trypanosomatid flagellates that inhabit the alimentary tract of insects. Several defined media have been developed, but it is of interest and very useful that the single medium of Steiger and Steiger (1976), devised for the promastigotes of *Leishmania donovani*, was found to support excellent growth of 3 species of *Leptomonas*, 4 of *Herpetemonas*, 6 of *Crithidia* and 1 of *Blastocrithidia*, all from insects (Fish et al. 1978). This same medium will also support growth of the plant flagellate *Phytomonas davidi* (only recently placed in culture — McGhee and Postell 1976) as well as of the promastigotes of *Leishmania tarentolae*, *L. braziliensis* and *L. donovani*. This medium therefore deserves to be looked at (Table 1). Cystine could be omitted as long as cysteine was present. Glutamic acid could also be omitted (Steiger and Steiger 1977) as well as serine. It is clear that we have here a medium for the study of com-

Table 1

Composition of Medium RE I (mg/l)

(A)	NaCl	8000	(C)	$NaHCO_2$	1000
	KCl	400		HEPES	14 250
	$MgSO_4 \times 7H_2O$	200	(D)	Adenosine	20
	$Na_2HPO_4 \times 2H_2O$	60		Guanosine	20
	KH_2PO_4	60	(E)	D-Biotin	1
	$CaCl_2$	70		Choline Cl	1
	Glucose	2000		Folic acid**	11
	Na-Acetate	600		i-Inositol	2
(B)	L-Arginine HCl	200		Niacinamide	1
	L-Cysteine HCl	50		D-Ca Pantothenate	1
	L-Cystine	50		Pyridoxal HCl	1
	L-Glutamic acid	300		Riboflavin	0.1
	L-Glutamine*	300		Thiamine HCl	1
	L-Histidine	100	(F)	Lipoic acid	0.4
	L-Isoleucine	100		Menadione	0.4
	L-Leucine	300		Vitamin A	0.4
	L-Lysine HCl	250	(G)	Ascorbic acid	0.2
	L-Methionine	50		Vitamin B_{12}	0.2
	L-Phenylalanine	100		Bovine Albumin (defatted)	15
	L-Proline	300			
	DL-Serine	200		Hemin**	10
	L-Threonine	400		Phenol red	10
	L-Tryptophan	50		Redist. H_2O Q.S.	1000 ml
	L-Tyrosine	50		pH adjusted with	
	L-Valine	100		1N NaOH to 7.3–7.4	

(From J. Parasitol. 62, p. 1010, 1976).

parative nutritional requirements of a range of lower trypanosomatids and of promastigotes of several species of *Leishmania*, including two important pathogens of man. Such a medium, and the similar medium of Berens and Marr (1978), prepared in part from commercially available solutions, lend themselves to detailed study of nutritional requirements, and some work of this sort has already been done with *L. donovani* promastigotes (Steiger and Black, 1980, Steiger and Steiger 1977). It is noteworthy that either proline or glucose could be omitted but not both, parallelling results of Krassner and Flory with *L. tarentolae* and again emphasizing the importance of proline in the metabolism of hemoflagellates (Bowman 1974, Trager 1974). *T. cruzi* has recently been grown in a defined medium at 27° using purified bovine liver catalase as source for both hemin and amino acids (Avila et al. 1979). Five different strains not only grew but also produced small numbers (5%) of infective trypomastigote forms. Other hemin-containing proteins could not be substituted for bovine liver catalase. Subunits of bovine liver catalase prepared by treatment with 6M urea still supported growth but an acid hydrolysate of the protein, or a mixture of its constituent amino acids, did not. Procyclic forms of certain strains of *T. brucei* have been grown in a nearly defined medium (Cross and Manning 1973) containing casein hydrolysate.

Among the insect hemoflagellates that have been grown in defined media are 3 species that harbor intracellular bacteria: *Crithidia oncopelti, C. deanei* and *Blastocrithiria culicis*. When *C. oncopelti* and *B. culicis* were rendered aposymbiotic, they could no longer be grown in defined medium; they now showed a requirement for a factor present in liver and as yet unidentified (Chang 1976). *C. deanei* freed of its symbiotes showed a similar requirement but this could be replaced with a high level of nicotinamide, 3 to 5 mg/100 ml as compared with the usual level in defined media of 1 to 5 mg/l (Mundim et al. 1977). Numerous other factors, including hemin, are supplied by the symbiotic bacteria, so that symbiote-containing strains can be grown in relatively very simple media (Mundim et al. 1974). The symbiotic bacteria have been shown to supply enzymes for heme synthesis in *C. oncopelti* and *B. culicis* (Chang et al. 1975) and of the urea cycle in *B. culicis* and *C. deanei* (Galinari and Camargo 1979).

The only group of parasitic protozoa other than trypanosomatids for which even nearly defined media have been available are the trichomonads (Shorb 1964, Honigberg 1978). These flagellates appear to have more complex nutritional requirements than the hemoflagellates. These include cholesterol, fatty acids, fat-soluble vitamins as well as the usual array of water soluble vitamins and amino acids. Attempts to

correlate nutritional factors with loss or recovery of pathogenicity would be of special interest for this group.

It is only recently that representative species of the symbiotic cellulose-digesting flagellates of termites and of the wood-feeding roach *Cryptocercus punctulatus* have been grown axenically (Yamin 1978, 1981), making possible detailed studies of their cellulose metabolism (Yamin 1980). *Trichomitopsis* was first obtained in axenic culture by the use of strict anaerobiosis and a medium supplemented with autoclaved rumen bacteria. The same methods were then successfully applied to other species including the hypermastigotes *Trichonympha spherica* and a *Trichonympha* from *Cryptocercus* (Yamin, personal communication). It seems possible that similar methods might permit axenic cultures of the rumen ciliates. Here many species of entodiniomorphids have been cultured with a mixed bacterial flora (Coleman 1979, Michelowski 1979) and *Entodinium caudatum* has been maintained in monoxenic culture for over 2 months (Hino and Kanetaka 1977). The holotrich rumen protozoa have proved difficult to culture but they have been kept going as mixed cultures in rumen fluid (Czerkawski and Breckenridge 1977).

As I mentioned at the beginning, there are two large groups of entirely parasitic protozoa, none of which have been grown axenically. These are the *Apicomplexa* and the *Microspora*. Many species spend all or part of their developmental cycle as parasites within other living cells. With the development of tissue culture methods, a number of species have been propagated in cell cultures. Several kinds of *Microspora* have been grown in insect cell cultures as well as in vertebrate cell cultures (Bismanis 1970, Undeen 1975). Whereas some species develop through only one cycle, from spore back to spore (Bismanis 1970), others show successive cycles. The latter is seen especially with *Nosema* (*Encephalitozoon*) *cuniculi*, a parasite of vertebrates (Pakes et al. 1975). With this species intracellular extrusion of the polar filament and sporoplasm were observed and this may account for the continuous propagation. Other propagative forms may, however, be present as in the hemolymph of silkworms infected with *N. bombycis* (Ohshima 1975).

The tachyzoite stage of *Toxoplasma gondii* (Dubey 1977) has long been grown in a number of different kinds of cell cultures, and this stage can be propagated indefinitely in this way. Such cultures have been used to study effects of drugs (Sheffield and Melton 1975), parasite-host cell interactions (Trager and Jensen 1976) and immunity (Jones et al. 1975), and parasite metabolic pathways (Pfefferkorn 1978). With other species of coccidia that lack, a continuo-

usly propagating asexual form, as in *Eimeria*, cell cultures support development of first generation merozoites from sporozoites and sometimes of second generation merozoites (Ruff and Reid 1977, Todd and Ernst 1977). For two species of *Eimeria* development through to oocysts has been obtained *in vitro*, first in *E. tenella* (Doran 1971) and then in *E. bovis* (Speer and Hammond 1973). It is highly interesting that gametogony of *Sarcocystis* with proof of its coccidial nature was first observed *in vitro* when Fayer (1972) inoculated bradyzoites (Dubey 1977), from *Sarcocystis* cysts in a grackle into cell culture. All of this serves to emphasize again the special value of cultures in relation to developmental cycles.

With several kinds of parasites of the Class *Sporozoea* in the *Haemosporina* and the *Piroplasmida* (Levine et al. 1980) portions of the life cycle have been grown in cell cultures *in vitro*. For the piroplasmids *Theileria parva*, the cause of East Coast fever of cattle, and *T. annulata*, cause of a less severe but still important disease, the macroschizonts have been cultured. These develop within lymphoblastoid cells transformed by the parasites and capable of continuous growth (Brown 1980). Division of the host cells depends on presence of the parasite. At cell division the parasites are distributed evenly to each daughter cell. Lymphoid cells from cattle can be infected *in vitro* with sporozoites from infected ticks; once infected, they are transformed and can then be propagated together with the macroschizonts within them. Such cultures have been used for a successful vaccination of cattle against *T. annulata* and as source of antigens for serodiagnostic and immunologic work. Microschizonts, the forms that give rise to the stage that infects the erythrocytes, have been produced in the cultures and infection of a small proportion of added red cells has been seen *in vitro* (Hulliger et al. 1966, Danskin and Wilde 1976 a, b). The piroplasms in erythrocytes are the presumed gametocytes that initiate a cycle in the vector tick, but *in vitro* development of this cycle has not yet been achieved.

With the other important group of piroplasmids, the Babesias, the situation appears superficially simpler. The erythrocytic stages of *Babesia bovis*, the most important babesia of cattle, have been grown continuously in cattle red cells (Erp et al. 1980), best results having been obtained with a static system under low O_2 tension (James et al. 1981) much like that used for *P. falciparum*. Material from the supernatants of such cultures has been used to immunize cattle (Smith et al. 1981). There is no evidence that the cultures are infective to ticks.

We come now to consideration of malaria parasites, from the standpoint of human disease, the most important members of this group of

protozoa. It is just five years since the first continuous culture of any malaria parasite. This was the human parasite, *Plasmodium falciparum* (Trager and Jensen 1976). Since then, the erythrocytic stages of four other species of malaria parasites, all from rhesus monkeys, have been cultured by the same methods. These are *P. knowlesi* (Chen et al. 1980, Wickham et al. 1980) with a 24-h cycle, *P. fragile* (Chin et al. 1979) a falciparum-like parasite, *P. inui* (Nguyen-Dinh et al. 1980) with a 72-h cycle, and a vivax-like parasite, *P. cynomolgi* (Nguyen-Dinh et al. 1981). In addition, there has been a report of the parasites of human quartan malaria *P. malariae* in mixed culture with *P. falciparum* (Rai Chowdhury et al. 1979). A report on the cultivation of *P. vivax* has just appeared (Larrouy et al. 1981). Essentially the same methods were used as for *P. falciparum* except for a higher level of glucose and change of medium three times per day.

For obvious reasons, most work continues to be done with *P. falciparum* and this parasite is now maintained in culture in a number of laboratories throughout the world. The cultures are being used to screen for antimalarials, to study drug resistance, to investigate host-parasite relations. They are providing information about the nature of the receptors on the host erythrocyte responsible for attachment by the merozoite, and why certain genetic red cell variants, as HbS, provide relative resistance to falciparum malaria. Most importantly, the cultures are being used in studies which we hope will lead to the identification of antigens responsible for protective immunity and in this way to a vaccine against malaria (Trager 1980).

We must remember that we have *in vitro* only one of the three cycles of development that comprise the complete life cycle of malaria parasites. This is the erythrocytic cycle. In this cycle in nature certain individual merozoites, for unknown reasons, develop into gametocytes rather than continuing the asexual cycle. These male and female gametocytes cannot develop further in the vertebrate host, but if ingested by a suitable mosquito, they produce gametes that initiate the sexual or sporogonic cycle. Formation of gametocytes was noted in the early cultures of falciparum. It was found that conditions deleterious to the asexual forms favored appearance of gametocytes and there is evidence for the involvement of cyclic AMP in this differentiation (Kaushal et al. 1980). In all early work, the gametocytes were non-infective to mosquitoes. Recently, however, gametocytes infective to mosquitoes have been regularly produced when hypoxanthine was added to the medium (Ifediba and Vanderberg, personal communication). Thus, the cultures can now provide a source of infected mosquitoes. Ookinete formation and partial development of already formed oocystes have been

obtained *in vitro*, but nothing approaching the full development from ookinete through oocyst to infective sporozoites (Schneider and Vanderberg 1980).

The sporozoites initiate the third developmental cycle of malaria. In bird malaria, the preerythrocytic or exoerythrocytic cycle occurs in endothelial cells. It has been propagated continuously in tissue cultures since the pioneer work of Huff (1964) (see Trager and Jensen, 1980). In mammalian malaria, however, the preerythrocytic cycle occurs in liver hepatic cells and is self-limited, that is, it does not repeat but must give rise to the erythrocytic cycle. Recently, this cycle has been obtained *in vitro* for the rodent malaria, *P. berghei* (Strome et al. 1979), using sporozoites placed in appropriate tissue cultures. It is interesting that *in vitro* there was no requirement for hepatic cells; the best cultures were in human embryonic lung cells. No similar development of a preerythrocytic stage of a human malaria parasite has yet been obtained, but there is now reason to hope that this can be done.

With these intracellular protozoa, as with other obligate intracellular parasites, there remains the most challenging problem of all — to grow them axenically, that is, without the host cell in a non-living medium. The erythrocytic stages of malaria may be supposed to be exceptionally favorable material for such work since they develop in a host cell that is no longer synthesizing protein. And indeed some extracellular development of the bird malaria, *P. lophurae*, has been obtained (Trager and Jensen 1980). Similar experiments with *P. falciparum* might be even more productive since more is known about the human erythrocyte than any other type of cell.

REFERENCES

Albright J. W. and Albright J. F. 1978: Growth of *Trypanosoma musculi* in cultures of murine spleen cells and analysis of the requirement for supportive spleen cells. Inf. Immun., 22, 343–349.

Avila J. L., Bretana A., Casanova M. A., Avila A. and Rodriguez F. 1979: *Trypanosoma cruzi*: Defined medium for continuous cultivation of virulent parasites. Exp. Parasit., 48, 27–35.

Baker J. R. 1966: Studies on *Trypanosoma avium*. IV. The development of infective metacyclic trypanosomes in cultures grown *in vitro*. Parasitology, 56, 15–19.

Berens R. L. and Marr J. J. 1978: An easily prepared defined medium for cultivation of *Leishmania donovani* promastigotes. J. Parasit., 64, 160.

Bingham A. K. and Meyer E. A. 1979: Giardia excystation can be induced *in vitro* in acidic solutions. Nature, 277, 301–302.

Bismanis J. E. 1970: Detection of latent murine nosematosis and growth of *Nosema cuniculi* in cell cultures. Can. J. Microbiol., 16, 237–242.

Bowman I. B. R. 1974: Intermediary metabolism of pathogenic flagellates. Ciba

Foundation Symposium 20. Trypanosomiasis and leishmaniasis. Elsevier North Holland, pp. 255–270.
Brown C. G. D. 1980: *In vitro* cultivation of *Theileria*. In: The *in vitro* Cultivation of the Pathogens of Tropical Diseases. Tropical Disease Research Series No. 3, Schwabe and Co., A. G., Basel, pp. 127–144.
Chang K.-P. 1976: Symbiote-free hemoflagellates, *Blastocrithidia culicis* and *Crithidia oncopelti*: Their liver factor requirement and serologic identity. J. Protozool., 23, 241–244.
Chang K.-P. 1980: Human cutaneous leishmania in a macrophage line: Propagation and isolation of intracellular parasites. Science, 209, 1240–1242.
Chang K.-P., Chang C. S. and Sassa S. 1975: Heme biosynthesis in bacterium—protozoon symbiosis: Enzyme defects in host hemoflagellates and complemental role of their intracellular symbiotes. Proc. Natl. Acad. Sci. U.S.A., 72, 2979–2983.
Chen Zhengren, Gao Minxin, Li Yuhua, Han Shumin and Zhang Nailin 1980: Studies on the cultivation of erythrocytic stage *Plasmoduim in vitro*, Chinese Med., J. 93, 31-35.
Chin W., Moss De Z. and Collins W. E. 1979: The continuous cultivation of *Plasmodium fragile* by the method of Trager-Jensen. Am. J. Trop. Med. Hyg., 28, 591–592.
Cleveland L. R. and Sanders E. P. 1930: Encystation, multiple fission without encystment, excystation, metacyclic development and variation in a pure line and nine strains of *Entamoeba histolytica*. Arch. Protistenk., 70, 223–266.
Coleman G. S. 1979: Rumen ciliate protozoa. In: Biochemistry and Physiology of *Protozoa*, (eds. M. Levandowsky and S. H. Hutner) 2nd edition, Vol. 2, Academic Press, New York, pp. 381–408.
Cross G. A. M. and Manning J. C. 1973: Cultivation of *Trypanosoma brucei* sspp. in semi-defined and defined media. Parasitology, 67, 315–331.
Cunningham I. and Taylor A. M. 1979: Infectivity of *Trypanosoma brucei* cultivated at 28° with tsetse fly salivary glands. J. Protozool., 26, 428–432.
Czerkawski J. W. and Breckenridge G. 1977: Design and development of a long-term rumen simulation technique (Rusitec). Brit. J. Nutrition, 38, 371–384.
Danskin D. and Wilde J. K. H. 1976a: Simulation *in vitro* of bovine host cycle of *Theileria parva*. Nature, 261, 311–312.
Danskin D. and Wilde J. K. H. 1976b: The effect of calf lymph and bovine red blood cells on *in vitro* cultivation of *Theileria parva* — infected lymphoid cells. Tropical Animal Health and Production, 8, 175–185.
Das S. R., Das P. and Rai G. P. 1979: Revival of pathogenicity of axenically grown *Entamoeba histolytica* for the rat. Austral. J. Exp. Biol. Med. Sci., 57, 241–244.
Diamond L. S. 1961: Axenic cultivation of *Entamoeba histolytica*. Science, 134, 336–337.
Diamond L. S. 1980: Axenic cultivation of *Entamoeba histolytica*: progress and problems. Arch. Invest. Med. (Mex.), 11 (Suppl. 1), 47–54.
Doran D. J. 1971: Increasing the yield of *Eimeria tenella* oocysts in cell cultures. J. Parasit., 57, 891–900.
Doyle J. J., Hirumi H., Hirumi K., Lupton E. N. and Cross G. A. M. 1980: Antigenic variation in clones of animal-infective *Trypanosoma brucei* derived and maintained *in vitro*. Parasitology, 80, 359–369.
Dubey J. P. 1977: *Toxoplasma, Hammondia, Besnoitia, Sarcocystis* and other tissue cyst-forming coccidia of man and animals. In: Parasitic Protozoa, (ed. Kreier J. P.), Vol. 3, Academic Press, New York, pp. 107–237.
El On J. and Greenblatt C. L. 1977: *Trypanosoma lewisi, T. acomys* and *T. cruzi*: A method for their cultivation with mammalian tissue. Exp. Parasit., 41, 31–42.
Erp E. E., Smith R. D., Ristic M. and Osomo B. M. 1980: Continuous *in vitro* cultivation of *Babesia bovis*. Am. J. Vet. Res., 41, 1141–1142.
Fayer R. 1972: Gametogony of *Sarcocystis* sp. in cell culture. Science, 175, 65–67.
Fish W. R., Holz G. G., Jr. and Beach D. H. 1978: Cultivation of trypanosomatids. J. Parasit., 64, 546–547.
Galinari S. and Camargo E. P. 1979: Urea cycle enzymes in wild and aposymbiotic strains of *Blastocrithidia culicis*. J. Parasit., 65, 88.
Gardiner P. R., Jones T. W. and Cunningham I. 1980: Antigenic ana-

lysis by immunofluorescence of *in vitro*-produced metacyclics of *Trypanosoma brucei* and their infections in mice. J. Protozool., 27, 316–320.
Giannini M. S. 1974: Effects of promastigote growth phase, frequency of subculture and host age on promastigote-initiated infections with *Leishmania donovani* in the golden hamster. J. Protozool., 21, 521–527.
Gray M. A., Brown C. G. D., Luckins A. G. and Gray A. R. 1979: Maintenance of infectivity of *Trypanosoma congolense in vitro* with explants of infected skin at 37°. Trans. Roy. Soc. Trop. Med. Hyg., 73, 406–408.
Gray M. A., Cunningham I., Gardiner P. R., Taylor A. M. and Luckins A. G. 1981: Cultivation of infective forms of *Trypanosoma congolense* from trypanosomes in the proboscis of *Glossina morsitans*. Parasitology, 82, 81–95.
Hill G. C., Shimer S. P., Caughey B. and Sauer L. S. 1978: Growth of infective forms of *Trypanosoma rhodesiense in vitro*, the causative agent of African trypanosomiasis. Science, 202, 763–765.
Hino T. and Kanetaka M. 1977: Gnotobiotic and axenic cultures of a rumen protozoan, *Entodinium caudatum*. J. Gen. App. Microbiol., 23, 37–48.
Honigberg B. M. 1978: Trichomonads of veterinary importance. Trichomonads of importance in human medicine. In: Parasitic Protozoa (ed. J. Kreier), Vol. 2, Academic Press, New York, pp. 163–273, 275–454.
Honigberg B. M. 1979: Biological and physiological factors affecting pathogenicity of trichomonads. In: Biochemistry and Physiology of *Protozoa* (eds. M. Levandowsky and S. H. Hutner) (2nd edition), Vol. 2, pp. 409–427.
Huff C. G. 1964: Cultivation of the exoerythrocytic stages of malarial parasites. Am. J. Trop. Med. Hyg., 13, 171–177.
Hulliger L. et al. 1966: Transition of developmental stages of *Theileria parva in vitro* at high temperature. Nature, 211, 328–329.
James M. A., Lev- M. G. and Ristic M. 1981: Isolation and partial characterization of culture-derived soluble *Babesia bovis* antigens. Inf. Immun., 31, 358–361.
Jones T. C., Len L. and Hirsch J. G. 1975: Assessment *in vitro* of immunity against *Toxoplasma gondii*. J. Exp. Med., 141, 466–482.
Kaushal D. C., Carter R. and Miller L. H. 1980: Gametocytogenesis by malaria parasites in continuous culture. Nature, 286, 490–492.
Lamy L. H. 1972: Protozoaires intracellulaires en culture cellulaire: Interet-possibilités-limites. Année Biol., 11, 145–183.
Larrouy G., Magnaval J.-F. and Moro F. 1981: A propos de l'obtention par culture *in vitro* de formes intra-erythrocytaires de *Plasmodium vivax*. C. R. Acad. Sci. Paris, 292, 929–930.
Levine N. D. et al. 1980: A newly revised classification of the *Protozoa*. J. Protozool., 27, 37–58.
Lycke E., Carlberg K. and Norrby R. 1976: Interactions between *Toxoplasma gondii* and its host cells: function of the penetration-enhancing factor of *Toxoplasma*. Inf. Immun., 11, 853–861.
Maramorosch K. and Hirumi H. (editors) 1979: Practical Tissue Culture Applications. Academic Press, New York.
McGhee R. B. and Postell E. J. 1976: Axenic cultivation of *Phytomonas davidi* (*Trypanosomatidae*), a symbiote of laticiferous plants. J. Protozool., 23, 238–241.
McHolland-Raymond L. E., Kingston N. and Trublood M. S. (1978): Continuous cultivation of *Trypanosoma theileri* at 37°C in bovine cell culture. J. Protozool., 25, 388–394.
Meerovitch E. and Ghadirian E. 1978: Restoration of virulence of axenically cultivated *Entamoeba histolytica* by cholesterol. Can. J. Microbiol., 24, 63–65.
Meyer E. A. 1970: Isolation and axenic cultivation of *Giardia* trophozoites from the rabbit, chinchilla, and cat. Exp. Parasitol., 27, 179–183.
Meyer E. A. 1976: *Giardia lamblia*: isolation and axenic cultivation. Exp. Parasitol., 39, 101–105.
Meyer H. and Xavier de Oliveira M. 1948: Cultivation of *Trypanosoma cruzi* in tissue cultures: a four year study. Parasitology, 39, 91–94.
Michelowski T. 1979: A simple system for continuous culture of rumen ciliates. Bull. Acad. Pol. Sci., (Sci Biol.), 27, 581–583.

Mitra S., Murti C. B. K. 1978: Encystation of axenically grown *Entamoeba histolytica*: effect of bacterial endotoxins, starch and epinephrine. Proc. Indian Acad. Sci., B. 87, 9–23.

Mundim M. H. and Roitman I. 1977: Extra nutritional requirements of artificially aposymbiotic *Crithidia deanei*. J. Protozool., 24, 329–331.

Mundim M. H., Roitman I., Herman M. A. and Kitajima E. W. 1974: Simple nutrition of *Crithidia deanei*, a reduviid trypanosomatid with an endosymbiont. J. Protozool., 21, 518–521.

Neva F. A., Malone M. F. and Myers B. R. 1961: Factors influencing the intracellular growth of *Trypanosoma cruzi in vitro*. Am. J. Trop. Med. Hyg., 10, 140–154.

Nguyen-Dinh P., Campbell C. C. and Collins W. E. 1980: Cultivation *in vitro* of the quartan malaria parasite *Plasmodium inui*. Science, 209, 1249–1251.

Nguyen-Dinh P., Gardner A. L., Campbell C. C., Skinner J. C. and Collins W. E. 1981: Cultivation *in vitro* of the vivax-type malaria parasite *Plasmodium cynomolgi*. Science, 212, 1146–1148.

Ohshima K. 1975: Propagative form of *Nosema bombycis* found in the hemolymph of silkworms. Annot. Zool. Japon., 48, 21–33.

Pakes S. P., Shadduck J. A. and Cali A. 1975: Fine structure of *Encephalitozoon cuniculi* from rabbits, mice and hamsters. J. Protozool., 22, 481–488.

Pan C. T. 1971: Cultivation and morphogenesis of *Trypanosoma cruzi* in improved liquid media. J. Protozool., 18, 556–560.

Pan S. C. T. 1978: *Trypanosoma cruzi*: Intracellular stages grown in a cell-free medium at 37°C. Exp. Parasit., 45, 215–224.

Pfefferkorn E. R. 1978: The enzymic defect of a mutant resistant to 5-fluorodeoxyuridine. Exp. Parasit., 44, 26–35.

Quadri S. S. 1962: The development in culture of *Trypanosoma striati* from an Indian fish. Parasitology, 52, 229–235.

Rai Chowdhury A. N., Chowdhury D. S. and Regis M. L. 1979: Simultaneous propagation of *P. malariae* and *P. falciparum* in a continuous culture. Indian J. Med. Res., 70 (Suppl.), 72–78.

Rengpian S. and Bailey G. B. 1975: Differentiation of *Entamoeba*: A new medium and optimal conditions for axenic encystation of *E. invadens*. J. Parasit., 61, 24–30.

Rowe D. S. and Hirumi H. (editors) 1980: The *in vitro* cultivation of the pathogens of tropical diseases. Tropical Diseases Research Series 3. UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases. Schwabe and Co., A. G., Basel.

Ruff M. D. and Reid W. M. 1977: Avian coccidia. In: Parasitic *Protozoa* (ed. J. P. Kreier), Vol. 3, Academic Press, New York, pp. 34–69.

Schneider I. and Vanderberg J. P. 1980: Culture of the invertebrate stages of plasmodium and the culture of mosquito tissues. In: Malaria, (ed. J. P. Kreier), Vol. 2, Academic Press, New York, pp. 235–270.

Sheffield H. G. and Melton M. T. 1975: Effect of pyrimethamine and sulfadiazine on the fine structure and multiplication of *Toxoplasma gondii*. J. Parasit., 61, 704–712.

Shorb M. S. 1964: The physiology of trichomonads. In: Biochemistry and Physiology of *Protozoa* (ed. S. H. Hutner), Vol. 3, Academic Press, N.Y., pp. 383–457.

Smith R. D., James M. A., Ristic M., Aikawa M., Vege y Murguia C. A. 1981: *Bovine babesiosis*: Protection of cattle with culture-derived soluble *Babesia bovis* antigens. Science, 212, 335–338.

Speer C. A. and Hammond D. M. 1973: Development of second generation schizonts, gametes and oocysts of *Eimeria bovis* in bovine kidney cells. Z. Parasitenk., 42, 105–113.

Sollard A. E. and Soulsby E. J. L. 1968: Cultivation of *Trypanosoma theileri* at 37°C in partially defined media. J. Protozool., 15, 463–466.

Steiger R. F. and Black C. D. V. 1980: Simplified defined media for cultivating *Leishmania donovani* promastigotes. Acta Tropica, 37, 195–198.

Steiger R. F. and Steiger E. 1976: A defined medium for cultivating *Leishmania donovani* and *L. braziliensis*. J. Parasit., 62, 1010–1011.

Steiger R. F. and Steiger E. 1977: Cultivation of *Leishmania donovani* and *L. braziliensis* in defined media: Nutritional requirements. J. Protozool., 24, 437–441.

Strome C. P. A., De Santis P. L. and Beaudoin R. L. 1979: Cultivation of the exoerythrocytic stages of *Plasmodium berghei* from sporozoites. *In Vitro*, 15, 531–536.
Taylor A. E. R. and Baker J. R. (editors) 1978: Methods of cultivating parasites *in vitro*. Academic Press, London.
Todd K. S., Jr. and Ernst J. V. 1977: Coccidia of mammals except man. In: Parasitic *Protozoa*, (ed. J. P. Kreier), Vol. 3, Academic Press, New York, pp. 71–99.
Trager W. 1974: Nutrition and biosynthetic capabilities of flagellates: Problems of *in vitro* cultivation and differentation. Ciba Foundation Symposium 20. Trypanosomiasis and leishmaniasis. Elsevier, North Holland, pp. 225–245.
Trager W. 1978: Cultivation of parasites *in vitro*. Am. J. Trop. Med. Hyg., 27, 216–222.
Trager W. 1980: Cultivation of malaria parasites *in vitro*: Its application to chemotherapy, immunology and the study of parasite-host interactions. In: The Host Invader Interplay, (ed. Van den Bossche), Elsevier, North Holland, pp. 537–548.
Trager W. and Jensen J. B. 1976: Human malaria parasites in continuous culture. Science, 193, 673–675.
Trager W. and Jensen J. B. 1980: Cultivation of erythrocytic and exoerythrocytic stages of plasmodia. In: Malaria, (ed. J. P. Kreier), Vol. 2, Academic Press, New York, pp. 271–319.
Undeen A. H. 1975: Growth of *Nosema algerae* in pig kidney cell cultures. J. Protozool., 22, 107–110.
Viens P., Lajeunesse M. E., Richards R. and Targett G. A. T. 1977: *Trypanosoma musculi*: *in vitro* cultivation of blood forms in cell culture media. Int. J. Parasitol., 7, 109–111.
Wickham J. M., Dennis E. D. and Mitchell G. H. 1980: Long term cultivation of a simian malaria parasite (*Plasmodium knowlesi*) in a semi-automated apparatus. Trans. Roy. Soc. Trop. Med. Hyg., 74, 789–792.
Yamin M. A. 1978: Axenic cultivation of the cellulolytic flagellate *Trichomitopis termopsidis* (Cleveland) from the termite *Zootermopsis*. J. Protozool., 25, 535–538.
Yamin M. A. 1980: Cellulose metabolism by the termite flagellate *Trichomitopsis termopsidis*. Applied and Environmental Microbiol., 39, 859–863.
Yamin M. A. 1981: Cellular metabolism by the flagellate *Trichonympha* from a termite is independent of endosymbiotic bacteria. Science, 211, 58–59.

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Invited Lecture

The Problem of Species and Intraspecific Variation in Protozoology

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There are certain biological problems which, having arisen early in the history of science did not lose their urgency up till now, and with their further development bacame filled with the new contents. The problem of species possess that character.

The category of species emerged originally from practical needs of the people to classify the surrounding animals and plants. As early as in the 18th century, it reached already a rather high level of development in works by Ray and Linnaeus who first introduced hierarchy of systematical units, proposed the delimitation of species as an invariable biological category, offered the Latin binary nomenclature. The great Darwin changed essentially the conventional view towards the living nature by establishing the basic laws of its evolution. However, the category of species still remained the principal notion, associated with the discrete development of life. And it is to the origin of species that the unfading book of Darwin was devoted.

The progress achieved in genetics in the first half of this century much stimulated and determined a new approach to the species problem. The complex and multiform genetic structure of species was defined, a notion of population as a form of species existence in various habitat conditions was put forward. Tschetwerikoff 1926), Dobzhansky (1937), Schmalhausen (1968) and others demonstrated that species having a sexual reproduction were rich in recessive mutations serving as "reserves" of hereditary variation (Schmalhausen 1968)

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to be realized upon changes in selection trends. The population genetics appeared and employing mathematics (Fischer 1930 and others) inserted some specific contents into the concept of natural selection which in Darwin's terms was of somewhat indefinite and descriptive character.

The problem of species formation (speciation) soon became experimental. To solve this problem, results of intensive botanical and zoological research were involved. This may be fairly exemplified by outstanding works of Dobzhansky and his students on speciation in *Drosophila* from tropical area of South America. In parallel with these studies into genetic processes, occurring within the species category, they originated a new and more strict concept of the species notion called the biological species concept, developed on botanical and zoological material (Dobzhansky 1937, Timofeyeff-Ressovsky et al. 1973, Mayr 1963, Vavilov 1922 and others). The essence of this concept being that the species differentiation is based on the phenomenon of their reproductive isolation, and the process of speciation involves the appearance of such an isolation. In his well-known book "Genetics and the Origin of Species" (1937) Dobzhansky regards species as a step of the evolutionary process: "... at which the once actually or potentially interbreeding array of forms becomes segregated in two or more separate arrays which are physiologically incapable of interbreeding" (p. 312).

And here is a bit different though similar in essence definition of species given in the recent interesting book by Génermont (1979) "The Mechanisms of Evolution". "Appartiennent à la même espèce tous les individus contemporains qui, pris deux a deux, ont, dans les conditions naturelles, une probabilité non nulle d'engendrer dans une generation ulterieure au moins un descendant commun fertile" (Génermont 1979, p. 12). See also papers by Génermont in the three-volume collective work published by the French Zoological Society "Les problèmes de l'espèce dans le régne animal" (1977–1980). I could make a long list of other definitions similar in essence. In the process of speciation, a new species may arise only after the establishment of the mechanism of reproductive isolation whose nature may be of various nature.

It goes on without saying that the elaboration of the biological concept of species reflected a great success in the elaboration of the problem of species as a whole. However, it does not settle this problem, since there are such forms of life — and at the very outset of life there were many such forms — in which the sexual process was lacking. Such a situation is now the case with many protozoa: amoebae, testacea, the majority of flagellates etc. There is no reason to believe that this ab-

sence might be of secondary nature. On contrary, I consider this to be the primary state for protozoa.

Thus, there is a number of protists lacking the sexual process and undergoing only asexual reproduction. A natural question arises — if the notion of species is applicable to these or they may constitute a peculiar, extra-specific form of life? Those who established and followed the biological concept of species deny the very applicability of the notion of species to the obligatory agamous protozoa. Here are several examples. Dobzhansky in his book cited above wrote: "The modification of the evolutionary patterns brought by the obligatory asexual reproduction and self-fertilization manifests itself in the absence of a definite species category in such organisms" (p. 319).

Similar view is shared by authors of the "biological concept of species". According to Génermont, "Il est evident, que chez ces êtres le mot speciation n'a aucun sens. Le mot espèce lui meme n'en a guere" (Génermont 1979, p. 45). Thus, in protozoa (more likely as in other organisms) there are two forms of life: the "specific" one (with the biparental sexual reproduction), and the "extra-specific" one (in protists with uniparental reproduction, including the obligatory agamous protozoa).

The standpoint of the authors of the "biological concept of species" just cited is to my mind erroneous and speaking more correctly, one-sided. I believe (and this view is in accord with views of many other biologists) that species, as a real natural phenomenon, is a general, universal form of life. However, its structure may be different at different steps of organic evolution. The specific form of life may be displayed variously, meaning that species are not equivalent. It is doubtless that each species is, according to Mayr, a "protected genofond", but this "protection" may be realized not only by the reproductive isolation whose great importance is beyond doubt. However, there are some other criteria of species that determine its essence, its nature, its very real existence as a definite and unique step in evolution. Among these criteria are morphological certainly ("hiatus" in relation to other species), geographical certainty, inner (populational) structure of species, its biochemical exclusiveness, its relative stability and integrity. At various steps of organic evolution, the "specific weight" of different criteria differs, the qualitative peculiarity of species being primarily determined by the form of reproduction.

The natural selection constitutes as a whole the main and determining factor of speciation in the organic evolution. It is due to natural selection and variety of environmental conditions that an "order" in organic life appears that ensures its most characteristic feature — its

discrete nature, which may be manifested irrespective of the reproductive forms. This is an unevitable consequence of the main factor able to transform the living nature — the natural selection.

Within *Protozoa*, three groups of species may be distinguished according to their mode of reproduction, their genetic nature and structure. The first one comprises the protozoa with an obligatory asexual reproduction. The second one involves haplonts with zygotic meiosis, i.e., sporozoans and numerous flagellates. The third group is constituted by diplonts with "biparental" reproduction and gametic meiosis, in combination with asexual reproduction and nuclear dualism. Here, primarily the ciliates belong. Let me consider, very shortly, the comparison of these groups.

As has been mentioned above, the reality of the species form of life in obligatory agamous protozoans is opposed by many followers of the "biological concept of species". Is this view correct? I do not think so, since the analysis of some specific evidence is suggesting the real existence of the species form of life in these obligatory agamous protists.

The problem of species in mammalian trypanosomes has been fairly elucidated in the H o a r e' s classical monograph (1972) who analysed a vast evidence so far available. H o a r e shows the reality of species existence (using numerous criteria), describes in detail geographical areas of these protozoans. Besides, a detailed analysis of the complex intraspecific differentiation is given. Of special interest are processes of microevolution and problems of speciation with trypanosomes. Different degrees of intraspecific differentiation are shown for various species of trypanosomes, these differences being explained most convincingly on the basis of the biology of species. H o a r e concludes: "Indeed, there are no grounds for assuming that organisms with agamous reproduction are less clearly demarcated by discontinuity than those with sexual reproduction" (p. 67) and adds: "Since these criteria satisfy the fundamental requirements, for populations to be recognized as species..." (p. 67).

Not less distinctively, than in trypanosomes, species are distinguished in other agamous parasitic protozoa, such as species of the genus *Entamoeba*. *E. histolytica* displays a complex intraspecific differentiation that involves various features of their morphology and physiology, and pathogenicity, in particular. The structure of species and intraspecific polymorphism with leishmania were recently examined in detail by B r a y (1969), S a f y a n o v a, and others. These aspects of research being not only of theoretical value, have also practical importance.

The really existing species are the cases of agamous free-living protozoa. Let me mention the early studies by J e n n i n g s (1916) on *Difflugia corona* showing the reality of the species and its complex

intraspecific differentiation. In my laboratory (Yudin 1970, Yudin and Sopina 1970 and Sopina 1973), studies into genetics and species structure of big amoebae of the type *Amoeba proteus* are in progress for several years. This species also displayed a complicated intraspecific differentiation that involves polymorphism of proteins, enzymes which were established using electrophoresis.

New possibilities and trends for analysis of intraspecific variation as well as of intraspecific distinctions in amoebae offers the "hybridization" technique used in transplantation of nuclei. Various degrees of compatibility of the nuclei and cytoplasm in different strains has been detected, and this suggests presumably a more or less pronounced degree of divergence of characters.

The reality of species in amoebae was recently confirmed by Friz (1974) who applied the "numeral taxonomy" technique for the taxonomy of amoebae. Regarding as many as 48 characters, he succeeded in finding clear distinctions between the three species of big amoebae of the proteus type.

Some doubt may appear, if the discussion of the presence or absence of the species form of life in agamous protozoa is in essence a vivid discussion of words? Is it really significant how to call various morphological forms? Not at all! The species is a really existing community of organisms, and its presence or absence would drastically change our concepts on evolutionary trends, on the character of microevolution, on the species formation. And it is no more chance that those who deny the reality of species in agamous protozoa escape in essence from regarding the trends of their evolution. Thus, Génermont, in his most interesting book covering 232 pages, devoted to the question of evolution of agamous protozoa only half a page! These animals seem to lay beyond the scope of the author's interest, although these are numerous enough to cover a large part of our planet. The present situation with the agamous protozoa should no longer remain as that. The problems of evolution and species formation in these protozoa must be paid a special attention. Real mechanisms underlying devergency and separation of the "protected genofonds" with the absence of sexual reproduction are to be looked for.

Of special interest should be the analysis of intraspecific variation. Here some questions arise, playing very likely no important role in the "biparentally" reproducing organisms, but being significant in "uniparentally" reproducing forms. I mean the epigenetic variation and long-lasting modifications.

In addition, a special attention should be paid to parasexual processes, which are almost unknown but may appear one of the forms of exchange of hereditary information within the asexually reproducing

species. This supposition is based on the evidence provided for *Entamoeba* by Enter (1971) who claimed the exchange of information between different strains without sexual process.

Of great interest in this respect may be the preliminary data on trypanosomes reported recently by Trait (1980) in "Nature". Using the electrophoresis technique, the author claimed the presence of recombination and segregation of hereditary characters. However, no sexual process, could be proved cytologically in trypanosomes. Of great interest are phenomena of "hereditary destabilization", reported from my laboratory (Yudin 1979, Makhlin and Yudin 1969), displayed during nuclear transplantation or other effects in *Amoeba proteus*.

Problems of genetics and microevolution in agamous protozoa seem to be of special urgency, their detailed elaboration constituting one of the trends in protozoan research in my laboratory.

Another form of species existence in protozoans is represented by haplonts with zygotic meiosis. The genetic structure of species here is qualitatively peculiar due to the presence in their genotype of only one allele of each locus throughout the larger part of the life cycle. With haplonts each mutation should have its phenotypical expression. During the latest decade, the number of theoretically and practically important publications appeared, dedicated to genetics and population genetics of *Sporozoa*. Some interesting evidence was provided by researchers from Edinburgh Genetical Institute (Walliker et al. 1975) on the genetics of *Plasmodium* species from rodents. A witty technique was proposed for strain hybridization that allowed to demonstrate a pronounced intraspecific polymorphism in respect to some proteins-enzymes. The hybridization experiments suggested the Mendelian segregation. The genetical polymorphism of species was reported also for *P. vivax* (Moshkovsky 1973, Lysenko 1977). The manifestation of such an important character as the time of incubation is presumably determined by a series of alleles different in various populations.

During the recent years much progress was achieved in the coccidian genetics (Jeffers 1978, and others). For some *Eimeria* species, a remarkable intraspecific polymorphism has been shown in respect to proteins-enzymes, to the duration of developmental stages, and to other markers. Thus, we observe the development of a new scientific field — the genetics of *Sporozoa*, which is of great importance for the problem of species and species formation in these organisms playing also significant role in medical and veterinary research.

Touching upon the third group of protozoa — the ciliates having gametic meiosis and nuclear dualism, with respect to the nature and structure of species, I must stress the great complication of the question of their species structure and forms of intraspecific polymorphism.

Genetics and species formation in ciliates are rather well elaborated. Recent studies by Sonnenborn (1957, 1975), Nanney (1980) and others are of special importance.

In ciliates the asexual reproduction is also dominating, local populations arising usually as a result of an intensive agamous multiplication. Adaptive modifications, including long-lasting modifications, are much involved in adaptations of populations to specific environmental conditions. It looks likely that epigenetic variation (a question open to further detailed examination) underlies the intraspecies polymorphism of ciliates. A peculiarity of the ciliate genome structure is constituted by the nuclear dualism and macronuclear polyploidy. Tweenspecies are presumably widely spread in ciliates, as reported by Sonneborn.

The species is a form of life existence common in all living organisms. On various steps of evolution the phenomenon of species bears its qualitative peculiarity. This is the case with protozoa especially in connection with their unicellularity, diversity of reproduction forms and complexity of their life cycles. To call attention of protozoologists to the urgency and necessity of elaboration of the problem of species in protozoa was the aim of my presentation.

REFERENCES

Bray R. 1969: Serotypes of *Leishmania* in relation to geography and disease states. In: Progress in Protozoology. Abstr. Third int. Congr. Protozool., Leningrad 1969, 288–289.

Dobzhansky Th. 1937: Genetics and the Origin of Species. New York, Columbia Univ. Press, 364 pp.

Entner N. 1971: "Mating" in *Entamoeba histolytica*. Nature, New Biol., 232–256.

Fisher R. 1930: The Genetical Theory of Natural Selection. Oxford, Clarendon Press.

Friz C. 1974: Taxonomic comparisons of *Amoeba proteus*, *Amoeba dubia* and *Chaos carolinensis*. I. Observations using principles of numeral taxonomy. Arch. Protistenk., 116, 236–243.

Génermont J. 1979: Les mécanismes de l'évolution. Dunod Université, Paris, 232 pp.

Hoare C. 1972: The Trypanosomes of Mammals. Oxford–Edinburgh, Blackwell Sci. Publishers 749 pp.

Jeffers T. 1978: Genetics of coccidia and the host response. In: Avian Coccidiosis. Edinburgh, 51–125.

Jennings H. 1916: Heredity, variation and the results of selection in the uniparental reproduction of *Difflugia corona*. Genetics, 1, 407–534.

"Les problemes de l'espece." t. I, 1976: t. II, 1977: t. III, 1980: Sous la direction de Ch. Boquet, J. Génermont, M. Lamotte. Memoires de Soc. zool. de France, No 38, 39, 40.

Lysenko A. 1977: Analysis of *Plasmodium vivax* from the angle of population biology. In: Protozoology, London, 3, 149–157.

Makhlin E. and Yudin A. 1969: [Incompatibility phenomena under nuclear transplantations in amoebae. I. The inviability of heterokaryons produced by implantation of the nucleus of *Amoeba proteus* into *Chaos chaos*]. Cytologija, 11, 744–759 (in Russian).

Mayr E. 1963: Animal Species and Evolution. Harvard Univ. Press, 797 pp.
Moshkovsky Ch. D. 1973: [The experience of interpretation of differences in incubation types and associated peculiarities in alternation of periods of acute fewer induced by various strains of *Plasmodium vivax*]. Med. Parazitol. Parazit. Bolezni, 11, 393–400 (in Russian).
Nanney D. 1980: [Experimental Ciliatology. An Introduction to Genetics Developmental] Analysis in Ciliates. New York, 304 pp.
Schmalhausen J. 1968: [The Factors of Evolution]. 2nd ed. Moscow, "Nauka" 451 pp. (in Russian).
Sonneborn T. 1957: Breeding systems, reproductive methods and species problem in *Protozoa*. In: The Species Problem. Washington, 155–324.
Sonneborn T. 1975: The *Paramecium aurelia* complex of fourteen sibling species. Trans. Am. Microsc. Soc., 91, 155–178.
Sopina V. 1973: [Esterases of three strains of *Amoeba proteus*]. Cytologija, 15, 1308–1312 (in Russian).
Trait A. 1980: Evidence for diploidy and mating in trypanosomes. Nature, 287, 536–538.
Timofeyeff-Ressovsky N., Yablokoff A. and Glotov H. 1973: [An Essay on the Problem of Populations]. Moscow, "Nauka", 277 pp. (in Russian).
Tschetwerikoff S. 1926: [On certain features of the evolutionary process from the viewpoint of modern genetics]. Zh. eksp. biol., 2, 3–54 (in Russian).
Vavilov N. 1922: The law of homologous series in variation. Genetics, 19, 47–77.
Walliker D., Garter R. and Sanderson A. 1975: Genetics studies on *Plasmodium chabaudi* recombination between enzyme markers. Parasitology, 70, 19–24.
Yudin A. 1979: Studies made by nuclear transplantation in *Amoeba proteus*. Int. Rev. Cytol., Suppl. 9.
Yudin A., Sopina V. 1970: On the role of nucleus and cytoplasm in the inheritance of some characters in amoebae (nuclear transfer experiments). Acta Protozool., 8, 1–39.

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Invited Lecture

In vivo Genetic Engineering in *Tetrahymena*

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Introduction

All sexual strains of ciliated protozoa have two different types of nuclei (Raikov 1976). The germinal micronucleus determines inheritance in the next sexual generation; the somatic macronucleus directs the phenotype of the cell. Much work has recently been done to compare the genetic constitution, function, and structure of these two nuclei. In *Tetrahymena thermophila* (formerly *T. pyriformis*, see Nanney and McCoy 1976), the micronucleus is diploid, with five pairs of chromosomes; the macronucleus contains about 24 times more DNA than the micronucleus (Woodward et al. 1972). They both have almost the same genetic complexity and chromatin structure, yet transcriptional activity, time of replication, and method and time of division are very different (Gorovsky 1973, Yao and Gorovsky 1974, Iwamura et al. 1979). In parallel with these molecular studies, a new genetic technology has developed which allows the independent manipulation of the two nuclei (Bruns and Brussard 1981). Since the macronucleus runs the cell, micronuclear genes (and chromosomes) can be eliminated. One application of this nuclear dimorphism is the engineering

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of strains with micronuclei which lack both copies of one (or more) of the chromosome pairs (nullisomics). This article will summarize how these strains were created, and present results of their use.

A basic understanding of ciliate genetics requires a knowledge of the genetic consequences of two previously described phenomena: conjugation, and genomic exclusion.

Conjugation

Conjugation occurs when two cells of different mating type are appropriately starved and allowed to form pairs (Bruns and Brussard 1974a). The resulting process may be divided into two parts: (1) the generation of a new, recombinant genome from the germ line of the conjugants, and (2) the differentiation of a new somatic nucleus with this new genome.

Production of a new genome involves meiosis in the micronucleus, with the elimination of all but one of the resulting haploid products in both members of each pair. The retained nucleus divides mitotically to form the genetically identical stationary and migratory haploid pronuclei. Each conjugant donates its migratory pronucleus to its mate; the two pronuclei then fuse to form the diploid fertilization nucleus (synkaryon). Thus the fertilization nuclei in the two members of any

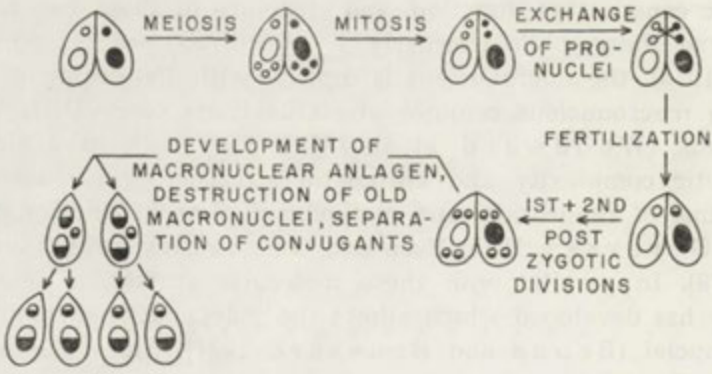

Fig. 1. Conjugation in *Tetrahymena thermophila*. See text for details. (From Bruns and Brussard 1974a)

pair are genetically identical. These nuclei then divide mitotically to form two different types of nuclei: macronuclear anlagen and micronuclei. The anlagen undergo DNA synthesis, initiate transcription, and develop into macronuclei before the cells resume vegetative growth. Figure 1 depicts this process.

Genomic Exclusion

This phenomenon, first described by Allen (1967 a and b), occurs when cells are induced to mate with one of several so called "star" strains (A*, C*). Figure 2 indicates that the non-star mate undergoes the normal events preceding fertilization (meiosis, elimination of three of the products, mitotic doubling of the retained haploid nucleus, and transfer of the migratory pronucleus). In contrast, the star member has a defective micronucleus which it loses at meiosis. After transfer, the only germinal genome in the two cells is that from the non-star parent. This nucleus undergoes an endoreduplication (Doerder and DeBault

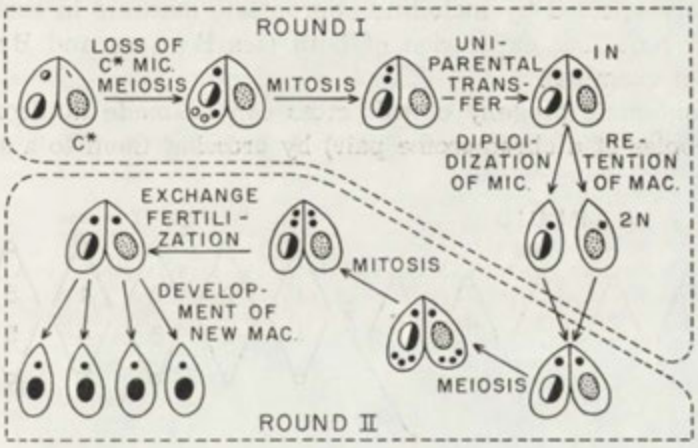

Fig. 2. Genomic exclusion. This example uses strain C*, but other "star" strains act the same. See text for details. (From Bruns and Brussard 1974 a)

1975), each conjugant now has an identical, fully homozygous genome. Thus the loss of the star strain micronucleus leads to uniparental micronuclear inheritance; the new genome is homozygous and comes only from the non-star parent. As Fig. 2 indicates, the next step in conjugation, development of the new macronucleus, fails to occur. The end result of this round of mating, called round I, is the generation, from each pair, of two cells with identical homozygous micronuclei derived solely from the non-star parent, but with parental macronuclei (see Ares and Bruns 1978 for a genetic analysis of the round I micronuclei). Thus a change in the germinal genome has been effected, but the cells' phenotypes (including mating type) are unaltered. These two cells can be cloned (the round I exconjugant clones) and remated. All events (fertilization and macronuclear development) occur normally in this second round of mating, termed round II. The phenotype expressed in the round II progeny reveals the genotype of the round I parents.

Producing Nullisomics

As has been described (Bruns and Brussard 1981) monosomics missing one copy of one or more chromosome can be created by crossing a diploid with a strain containing a haploid micronucleus. The haploid strain is generated by crosses in which three, instead of two, cells mate (Preparata and Nanney 1977). The triplet yields three cells with equivalently sized macronuclei, but micronuclei which are haploid, diploid, and triploid, respectively. All three strains, including the haploid, can be induced to mate. Upon mating, the haploid attempts meiosis, which, not surprisingly, generally produces pronuclei missing one or more of the chromosomes. Progeny of the haploid-diploid cross can be easily selected by including appropriate markers in each parental strain, and requiring expression of both (see Bruns and Brussard 1981, for an example).

The monosomic progeny of this cross can be made nullisomic (missing both copies of a chromosome pair) by crossing them to a star strain.

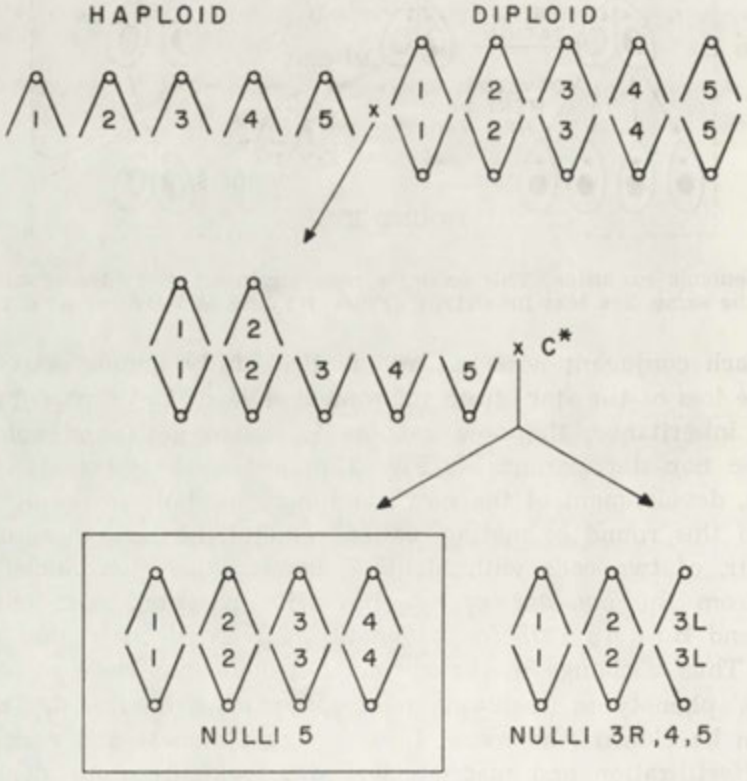

Fig. 3. Haploid by diploid cross, followed by genomic exclusion. Each of the five metacentric chromosomes is represented and numbered; only micronuclear karyotypes are indicated. The strain used as a standard is enclosed in a box

Since genomic exclusion involves meiosis and the endoreduplication of some resulting meiotic product, the exconjugants should be either nullisomic or diploid for each of the chromosome pairs.

Figure 3 indicates the micronuclear events that occurred when the haploid was crossed with a diploid. Although the progeny were probably monosomic for chromosomes 3, 4, and 5, the homologous chromosomes pair so tightly during metaphase I, that it is difficult to determine the degree of monosomy by cytology. On the other hand, crossing to C* yielded genomic exclusion progeny which included a strain missing both copies of the smallest chromosome (chromosome 5, see Bruns and Brussard 1981), and a strain missing both copies of chromosomes 4 and 5 plus the right arm of chromosome 3. As Fig. 4 indicates, the mul-

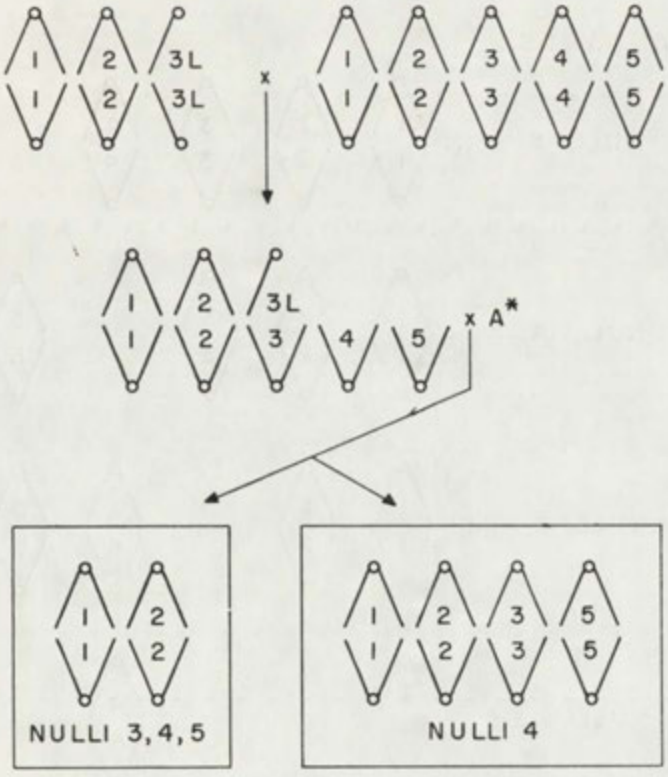

Fig. 4. Nulli 3R, 4, 5 by diploid cross followed by genomic exclusion. The strains used as standards are boxed

tiple nullisomic was crossed with a diploid to make a multiple monosomic, and again passed through genomic exclusion. This time a multiple nullisomic, missing chromosomes 3, 4, and 5, and a single nullisomic, missing chromosome 4, were isolated. It should be emphasized

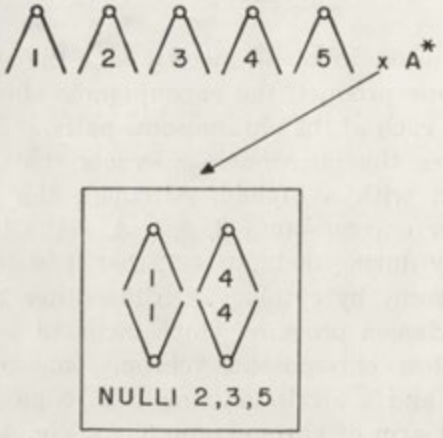

Fig. 5. Haploid by A* cross to yield the standard shown in the box

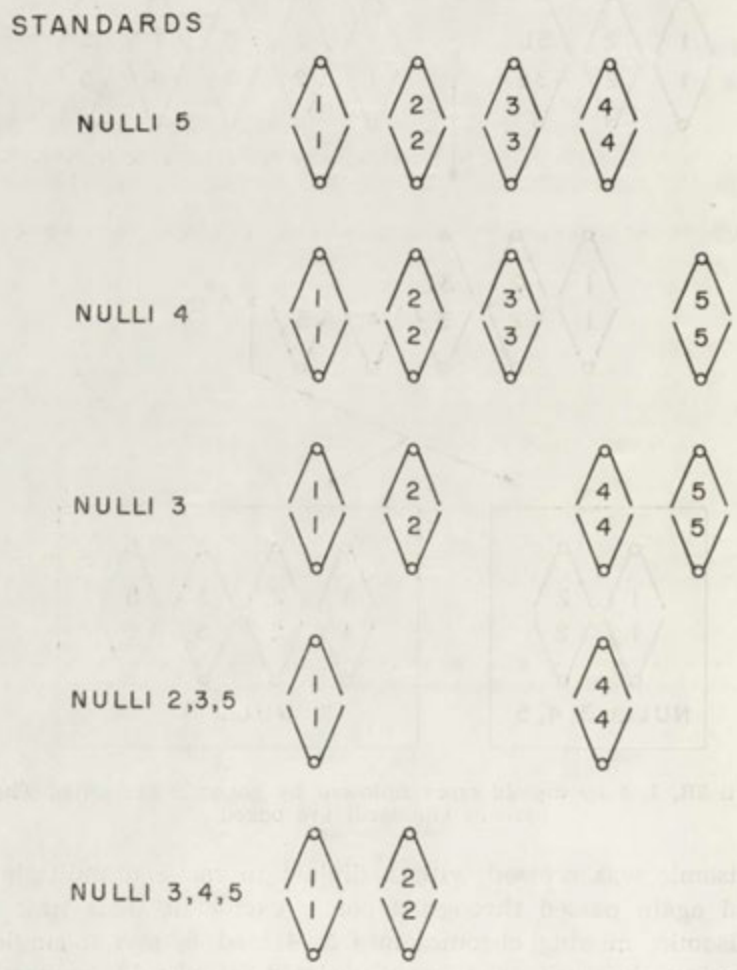

Fig. 6. Micronuclear chromosomal constitution of the set of nullisomics used as standards

that all these nullisomics are viable because the parental macronucleus is retained during genomic exclusion. The 3, 4, 5 nullisomic was again made monosomic by a cross to a diploid, and again taken through genomic exclusion. A strain missing both copies of chromosome 3 was isolated.

An alternate method to generate nullisomics is indicated in Fig. 5. In this approach, the haploid is mated with a star strain, and progeny retained. Most progeny of this cross died (they probably become amicronucleate); a few survived. From these we isolated a clone with the haploid macronucleus, but a micronucleus missing both copies of three of the chromosomes (Nulli 2, 3, 5).

Figure 6 diagrams the micronuclear constitution of the strains which we use as standards. Every chromosome but number 1 is eliminated in at least one strain. As will be described below, crosses with this set of standards allows mapping of genes to chromosomes. Figure 7 shows the karyotype of two of the nullisomics. The nulli 5 has only four chromosome pairs, and the nulli 3, 4, 5 has only two.

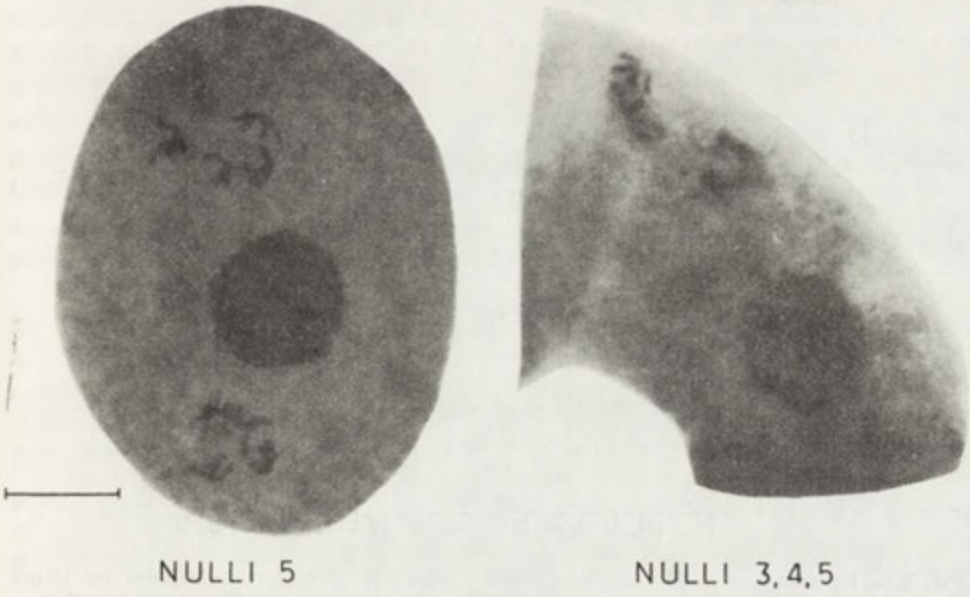

Fig. 7. Karyotypes of the Nulli 5 and Nulli 3, 4, 5 shown in Fig. 6. Staining done as in Bruns and Brussard (1981), except preparations were stained immediately after fixing (even a few hours in the 70% ethanol results in poor spreading of the chromosomes). The bar indicates 10 micrometers

This set of nullisomics has provided information in several studies. The rest of this paper will briefly describe these observations.

Nullisomics and the Crescent

During an early stage of meiosis the micronucleus stretches out to form one long filament, called the crescent (R a y 1956). Figure 8 A is this stage in a cross of two diploids; the striking synchrony in elongation of the two members of the pair is typical. Both R a y (1956) and S u g a i and H i w a t a s h i (1974) suggest that this structure is caused by an unwinding of the chromosomes, with the five pairs of chromosomes linked end to end, possibly in a specified order.

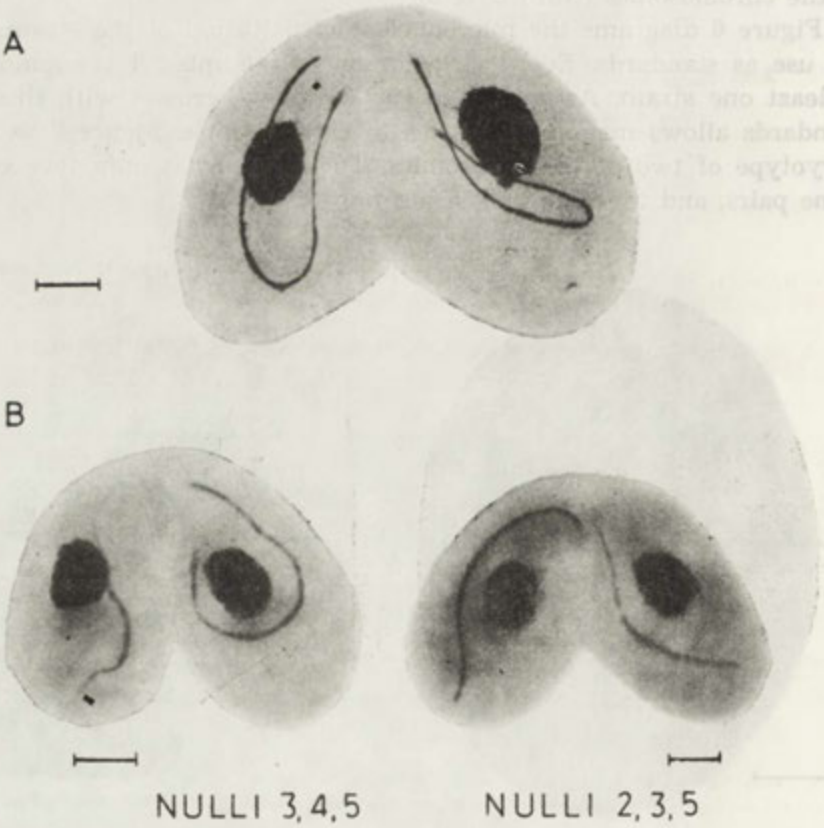

Fig. 8 A. Crescent in a diploid by diploid cross. 8 B. Crescent in diploid by triple nullisomic crosses. Staining was as for Fig. 7. The bar equals 10 micrometers

Crosses to nullisomics shed new light on this structure. Each of the three single nullisomic strains was examined during meiosis; each had a single crescent. This must mean that there is no specific order for chromosome end to end alignment, since one of the three strains would have to be missing a chromosome which is not on one or the other end

of the crescent. In addition, the two triple nullisomic strains were crossed to diploids, and the crescents examined. Figure 8 B shows the crescents from these crosses. Both triple nullisomics yield full length but faintly staining crescents. Bearing in mind that each of the nullisomic strains contains only two out of the normal complement of five pairs of chromosomes, these results demonstrate that length of the crescent cannot be a simple function of the total length of end to end associated chromosomes. This result is much more in keeping with the conclusions of Wolfe et al. (1976); the crescent may be interphase chromatin pulled into the long structure by microtubules which form under the nuclear envelope inside the micronucleus.

Mapping with Nullisomics

As introduced in Bruns and Brussard (1981), nullisomics can be used for gene mapping. A nullisomic crossed to a diploid yields monosomic progeny. The macronucleus of *Tetrahymena* is apparently able to cope with extreme genetic imbalance; single, double, and even triple monosomic progeny with new (non parental) macronuclei are viable. For mapping, a homozygote for the gene in question is crossed to the nullisomics. In any given cross, if the gene being mapped is on the chromosome that is missing in the nullisomic, the progeny will have only one copy of the gene (i.e., are hemizygous) and will express the mutant phenotype. If the gene is on a chromosome that is present in the nullisomic, the progeny will get a copy of the chromosome from each parent, be heterozygous, and initially express the dominant phenotype.

If the mutation being mapped is dominant, both a hemizygote and a heterozygote will initially have the same phenotype. This is not a problem since *Tetrahymena* undergoes phenotypic assortment, a phenomenon whereby heterozygotes assort vegetative progeny expressing the phenotype of only one of the two alleles (see Sonneborn 1975 for a review). Heterozygotes will assort cells that express the recessive phenotype (see Bruns and Brussard 1974 b for other applications of this phenomenon). Therefore, whether the mutation being mapped is dominant or recessive, hemizygous progeny will have a different phenotype than heterozygotes. Together with M. Altschuler, L. Martin, E. Orias, M. Flacks, M. Baum, F. P. Doerder, and L. Bleyman we have mapped over 70 mutations. Additionally, in cooperation with M.-C. Yao (in preparation), we have mapped the location of the ribosomal RNA gene by hybridizing a radioactive cloned rDNA sequence to DNA from isolated nullisomic micronuclei. Only strains missing chromosome 2 lack micronuclear rDNA. Table 1 summarizes the findings.

Table 1

Chromosome Assignments

Chromosome	Number of Temperature Sensitives	Number of Nutritional Auxotrophs	Antimetabolite Resistant	Other
1	5	6	*gal, ChxA*	*ts-101*
2	10	3	*Pmr, Mpr*	*Mat, rDNA*
3	8	1	—	*pig-1*
4	4	9	—	*SerH*
5	8	—	—	*rseB, RseD*
				6 X-ray induced lethals

A listing of the number of temperature sensitive and nutritional auxotrophic mutations assigned to each of the chromosomes. Antimetabolite resistant and other markers are listed individually. Most of the named markers are listed in Bruns and Orias 1980). Previously unnamed markers are *Pmr* = resistance to paramomycin; *ts-101* = temperature sensitive which phenotypically coassorts with *gal*; *pig* = tyrosine dependent pigment producer (E. Orias, personal communication); X-ray induced lethal mutations, probably deletions (M. Altschuler, personal communication).

Homozygous Deletions

The triple nullisomic depicted in Fig. 5 was crossed to a diploid to produce a triple monosomic. This line was crossed to strain A*, and exconjugants cloned. We retained clones with the following properties: (1) they could not yield progeny when the two round I clones were mated for round II genomic exclusion, and (2) they could yield progeny when crossed to diploids. The clones were examined cytologically and genetically.

The number of chromosomes in each of the round I clones was determined in crosses with strain A*, by adding 2 μg/ml cycloheximide to the matings during crescent phase of meiosis, and fixing for staining 2 1/2 h later. As described by Kaczanowski (1981), this treatment blocks conjugation in metaphase, simplifying chromosome counts. A list presenting the number of chromosomes observed in each strain is on the right side of Fig. 9.

The strains were examined genetically by crossing each to strains homozygous for mutations which had already been mapped to specific chromosomes, as described in a previous section of this paper. Remarkably, all of the stocks listed in Fig. 9 act genetically as if they are missing parts of chromosomes. For example, strains CU374, 375, and 376 all uncover the same subset of chromosome 2 markers. Similarly CU370, 371, and 372 uncover one chromosome 1 marker, whereas strain CU373 uncovers the other chromosome 1 markers. We assume that these strains have lost whole chromosome arms during the genomic exclusion cross,

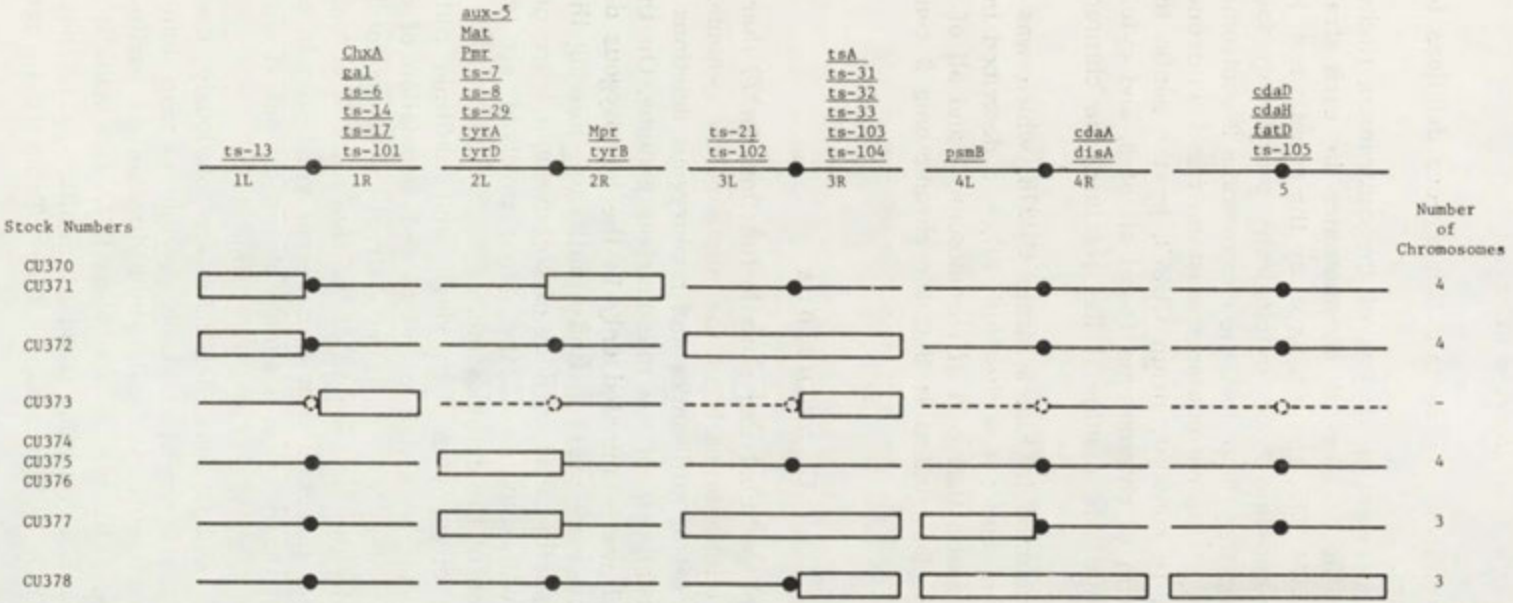

Fig. 9. Homozygous deletion strains. The five chromosomes are indicated, with centromere in the center. The boxes on the chromosomes indicate where they are deleted. The dashed lines for CU373 indicate what has not been tested. The column on the right lists the number of chromosomes seen cytologically for each strain. The mutations labeled ts are temperature sensitives of various origins. The aux mutation is an undefined nutritional auxotroph (E. Orias, personal communication). Other mutations are listed in Bruns and Orias (1980), or the legend of Table 1

and that their micronuclei now contain homozygous deletions for large parts of chromosomes.

We have assumed retention or loss of the centromere (indicated in Fig. 9) to reconcile the number of chromosomes in each strain. For example, strains CU374, 375, and 376 have, by the genetic test, lost only the left arm of chromosome 2, yet cytologically reveal only four chromosomes. We assume they have lost the chromosome 2 centromere, and the retained portion of the chromosome must be fused with one of the other chromosomes. In contrast, strain CU372, by the gentic tests, has lost both the left arm of chromosome 1 and all of 3, and cytologically has four chromosomes; we assume it has retained the chromosome 1 centromere.

We have also included in Fig. 9 strain CU378, which was derived from a different monosomic; it is the Nulli 3R, 4, 5 described in Fig. 3. The genetic tests indicate that part of chromosome 3 and all of 4 and 5 are missing. The cytology indicates that the chromosome 3 centromere was retained.

Conclusions

The separation of germinal and somatic functions in *T. thermophila* has allowed us to eliminate parts of the micronuclear genome. Taken together, the whole set of nullisomics and homozygous deletions account for almost, and possibly all, of the micronuclear genome. On the other hand, chromosome 1 was eliminated only in the homozygous deletions, even though attempts were made to find a nullisomic missing the whole chromosome. It is possible that portions of chromosome 1 are necessary for vegetative growth, but if so, the genes involved are probably clustered in a portion of the chromosome.

Analysis of the crescent in both single and multiple nullisomics indicates that it cannot be a simple end to end association of chromosomes with a fixed order. Nor can it be a structure whose total length is normally limited by the total length of the five pairs of chromosomes. A simple view is that the chromosomes have not yet begun to condense for meiosis, and that the crescent is simply chromatin pulled out by the microtubules described by Wolfe et al. (1976).

Nullisomics and homozygous deletions are obviously useful for mapping. Phenotypic assortment makes it possible to map dominant as well as recessive mutations. Molecular hybridization of purified DNA sequences to purified nullisomic micronuclear DNA has made it possible to map genes without performing standard matings as is shown here for the ribosomal DNA on chromosome 2 (see Table 1). In agreement

with the chromosome arm assignment, a genetic analysis has shown meiotic linkage of three of the genes assigned to the same arm of chromosome 3 (Merriam and Bruns, in preparation).

Finally, the mechanism creating the homozygous deletions is not known. Each was isolated after meiosis in a multiple monosomic, but the production of deletions after meiosis in diploids, single monosomics, or nullisomics has not been systematically studied. It may be that illegitimate pairing, and possibly recombination, is promoted in the multiple monosomic strains and can lead to the loss of whole arms of chromosomes.

REFERENCES

Allen S. L. 1967 a: A rapid means for inducing homozygous diploid lines in *Tetrahymena pyriformis*, syngen 1. Science, 155, 575–577.

Allen S. L. 1967 b: Cytogenetics of genomic exclusion in *Tetrahymena*. Genetics, 55, 797–833.

Ares M. Jr. and Bruns P. J. 1978: Isolation and genetic characterization of a mutation affecting ribosomal resistance to cycloheximide in *Tetrahymena*. Genetics, 90, 463–474.

Bruns P. J. and Brussard T. B. 1974 a: Pair formation in *Tetrahymena pyriformis*, an inducible developmental system. J. Exp. Zool., 188, 337–344.

Bruns P. J. and Brussard T. B. 1974 b: Positive selection for mating with functional heterokaryons in *Tetrahymena pyriformis*. Genetics, 78, 831–841.

Bruns P. J. and Orias E. 1980: *Tetrahymena*. In: Genetic Maps (ed. S. J. germinal chromosomes. Science, 213, 549–551.

Bruns P. J. and Orias E. 1980: *Tetrahymena*. In: Genetic Maps, (ed. S. J. O'Brien), National Cancer Institute, Bethesda, pp. 139–141.

Doerder F. P. and deBault L. E. 1975: Cytofluorimetric analysis of nuclear DNA during meiosis, fertilization, and macronuclear development in the ciliate *Tetrahymena pyriformis*, syngen 1. J. Cell Sci., 17, 471–493.

Gorovsky M. A. 1973: Macro- and micronuclei of *Tetrahymena pyriformis*: a model system for studying structure and function of eukaryotic nuclei. J. Protozool., 20, 19–25.

Iwamura Y., Sakai M., Mita T. and Muramatsu M. 1979: Unequal gene amplification and transcription in the macronucleus of *Tetrahymena pyriformis*. Biochem., 18, 5289–5294.

Kaczanowski A. 1981: Cell interaction during conjugation of cycloheximide resistant and cycloheximide sensitive cells in *Tetrahymena thermophila* in pairs treated with cycloheximide. Progress in Protozoology, Abstracts, 165.

Nanney D. L. and McCoy J. W. 1976: Characterization of the species of the *Tetrahymena pyriformis* complex. Trans. Am. Microsc. Soc., 95, 664–682.

Preparata R. M. and Nanney D. L. 1977: Cytogenetics of triplet conjugation in *Tetrahymena*: origin of haploid and triploid clones. Chromosoma, 60, 49–57.

Raikov I. B. 1976: Evolution of macronuclear organization. Annual Rev. Genetics, 10, 413–440.

Ray C. Jr. 1956: Meiosis and nuclear behavior in *Tetrahymena pyriformis*. J. Protozool., 3, 88–96.

Sonneborn T. M. 1975: *Tetrahymena pyriformis*, In: Handbook of Genetics, (ed. R. C. King) Plenum Press, New York, Vol. 2, pp. 433–467.

Sugai T. and Hiwatashi K. 1974: Cytological and autoradiografic studies of the micronucleus at meiotic prophase in *Tetrahymena thermophila*. J. Protozool., 23, 542–548.

Woodward J., Kaneshiro E. S. and Gorovsky M. A. 1972: Cytochemical studies on the problem of macronuclear subnuclei in *Tetrahymena*. Genetics, 70, 251–260.

Wolfe J., Hunter B. and Adair W. S. 1976: A cytological study of micronuclear elongation during conjugation in *Tetrahymena*. Chromosoma, 55, 289–308.

Yao M.-C. and Gorovsky M. A. 1974: Comparison of the sequences of macro- and micronuclear DNA of *Tetrahymena pyriformis*. Chromosoma, 48, 1–18.

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Invited Lecture

Microtubule-organizing Centres and Microtubule-deployment in *Protozoa*

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Summary

This article deals with recent advances in our understanding of the extent to which microtubule-organizing centres (MTOCs) organize microtubules during microtubule assembly and deployment in *Protozoa*. Attention is drawn to the phenomenon of linear microtubule-differentiation because it is an organizational achievement that is probably not under direct MTOC jurisdiction. A multi-nucleating-element hypothesis is advanced to account for control of the assembly of microtubules with different compositions and properties.

Investigations of the assembly of highly-ordered microtubule arrays in the heliozoan *Echinosphaerium* and the ciliate *Nassula* reveal that certain MTOCs differ in the amount and/or quality of spatial information that they provide. It is argued that these differences may be related to the various ways in which information stored in the molecular fabric of microtubule walls is exploited for different aspects of microtubule differentiation after microtubules have grown out for considerable distances (several μm) from MTOCs. Studies of micronuclear spindle microtubules in *Paramecium*, suctorian tentacle organization, and basal body morphogenesis are cited in support of these ideas. Substantial indications that mechanisms for control of microtubule differentiation, number, orientation, polarity and positioning in protozoans provide important paradigms for those investigating MTOC function and microtubule deployment in multicellular organisms is emphasized.

In Memoriam

This article is dedicated to the memory of Tracy Morton Sonneborn. Earlier this year the science of protozoology lost one of its most distinguished and penetrating contributors.

During the presentation of a paper at the Second International Conference on Protozoology (which was held in London in 1965), Professor Sonneborn pointed

Paper prescented on Plenary Session on July 5 at VI International Congress of Protozoology, Warszawa, Poland, 5–11 July 1981.

out that discoveries of fundamental importance for biology in general had emerged from studies of *Protozoa*. He forecast that this would continue to be the case. These particular remarks were not published in the conference proceedings. It is pertinent to recall them now because in the sixteen years that have intervened *Protozoa* have yielded valuable information of widespread significance concerning MTOC capabilities, microtubule deployment, and cell morphogenesis. Tracy Sonneborn, and many of those who have had the privilege of working with him, have made a number of fundamental contributions in this area (for example, the reports authored by Aufderheide, Beisson, Dippell, Grimes, Nanney, and Sonneborn, that are cited below). It is also an area that is being explored with great success here in Poland (for example, the reports authored by Bąkowska, Golińska and Kink, and Jerka-Dziadosz, that are cited below). Hence, Warsaw/1981 is a very appropriate place and time to salute Tracy Sonneborn.

Introduction

The assembly of many, perhaps all, of a eukaryotic cell's microtubules is nucleated at one or more discrete sites. Twelve years ago Pickett-Heaps (1969) suggested that these sites be referred to as microtubule-organizing centres. This term has been widely adopted but the ways in which MTOCs organize microtubules is only just beginning to become apparent. Examinations of the assembly of highly-ordered microtubule arrays in certain protozoans have been especially instructive.

In a few cases there is exact specification of the number of microtubules that grow out from an MTOC. Because of this, it has been suggested that each MTOC includes a number of nucleating elements and that each nucleating element initiates the assembly of one microtubule (Tucker 1977). The molecular nature of nucleating elements remains to be ascertained but there is little doubt that *in vivo* they are usually essential for the nucleation of microtubule assembly.

The wall of a microtubule consists largely of protein heterodimers called tubulins and is usually constructed as outlined below (see Amos 1979). Tubulins are arranged in longitudinally oriented rows called protofilaments (Fig. 1). In most cases a wall includes 13 protofilaments. The portions of tubulins that face onto the outer surface of a microtubule wall have a bilobular topography. Each lobe probably reveals the position of an α or a β tubulin monomer in a heterodimer. Furthermore, microtubules have a distinct polarity. In most cases all a microtubules' tubulins probably have the same orientation with respect to the longitudinal axes of a microtubule and its protofilaments. This polarity may be specified by nucleating elements. For example, microtubule doublets in cilia of the ciliate *Tetrahymena*, and axonemal microtubules in the heliozoan *Echinosphaerium*, are both constructed with the same polarity.

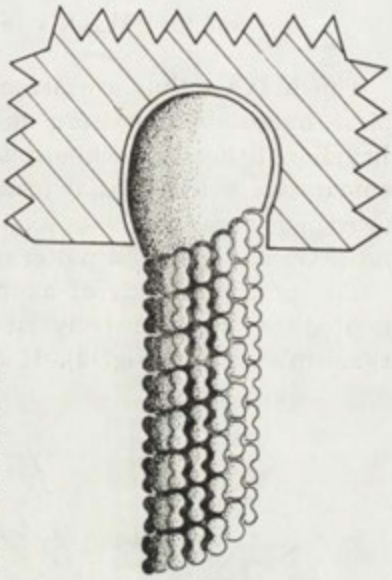

Fig. 1. Schematic diagram showing the helico-longitudinal arrangement of tubulin dimers in the proximal portion of a microtubule. The minus end of the microtubule where assembly starts is capped by a nucleating element that is partly embedded in a portion of the dense material (cross-hatched) of an MTOC. The bilobular topography of the outer surfaces of tubulin dimers and the longitudinal orientation of protofilaments are also shown

The plus ends (fast-growing ends) of these microtubules are directed away from the MTOCs and nucleating elements that initiate their assembly (Euteneuer and McIntosh 1981). However, it is not yet known if tubulins are oriented with their α tubulins pointing towards the plus end of a microtubule or *vice-versa*.

A range of protein species that are collectively referred to as microtubule-associated proteins (MAPs) also contribute to the structure of microtubule walls. Full details of the ways in which they are included in, or bound to, the tubulin fabric are not yet available. At least one type of MAP includes a component that projects out for several nanometers from the outer wall surface and forms a regular lattice over this surface. Portions of this MAP, and perhaps other MAPs, may be situated in the longitudinally and helically oriented grooves that run between adjacent protofilaments and tubulin dimers (see Fig. 1).

Several MTOCs appear to consist mainly of amorphous dense material when examined using electron microscopy. This material is arranged in a variety of configurations depending on the MTOC in question. Much of this material may be involved in anchoring an MTOC's nucleating elements. In some cases it may represent a compact highly-ordered fabric that binds nucleating elements in precisely defined positions at particular orientations (Fig. 1). In these instances MTOCs could, at least in theory, specify microtubule spacing, orientation, number and polarity (Tucker 1979). Analyses of microtubule morphogenesis in certain protozoans leave little doubt that these potentialities are sometimes realised.

Axonemal MTOCs in the Heliozoan *Echinosphaerium*

The MTOCs that are associated with the generation of the double-spiral microtubule pattern in the axopodial axonemes of the actinophryidian heliozoan *Echinosphaerium nucleofilum* apparently contribute comparatively few spatial instructions during pattern formation. This is surprising because these axonemes exhibit one of the most complex and precisely specified patterns of microtubule packing described so far.

The proximal ends of axonemes in this multinucleate heliozoan are situated in the cell body at a level close to that occupied by the organism's nuclei (Fig. 2). If cultures of the organisms are kept in an

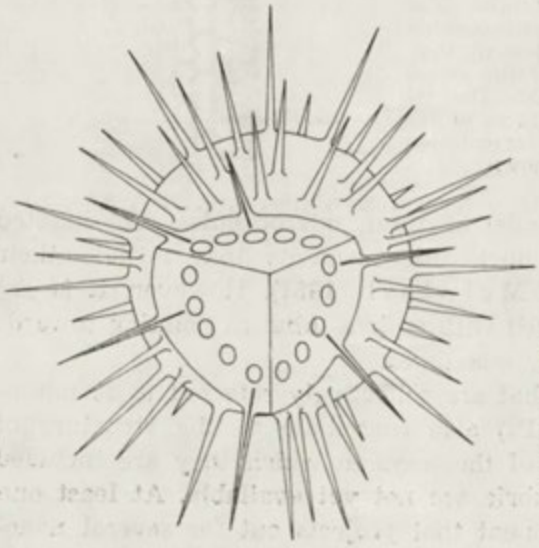

Fig. 2. Schematic diagram of *Echinosphaerium* to show the way in which its numerous tapering axopodia radiate from the surface of the cell body. A sector has been removed to show how nuclei are all positioned at about the same level close to that at which the proximal ends of axonemes are situated

incubator set at 0°C for 6 h all the axopodia retract and nearly all the axonemes disassemble completely. Irregularly shaped clumps of dense material are situated near the nuclei after this procedure. Each clump acts as an axonemal MTOC and initiates assembly of microtubules for one new axoneme (Jones and Tucker 1981). Within 7 min of transfer of cultures to room temperature (20°C) large numbers of microtubules start to grow out in an apparently random variety of directions from the MTOC clumps (Fig. 3). In this case the MTOC does not seem to specify the orientation and spacing of microtubules with any great precision at all. These specifications are apparently effected by a self-linkage procedure (Tucker 1977) when intertubule links join the microtubules together.

Axonemal microtubules are differentiated from each other in terms of the number of links that connect them to their neighbours. For ex-

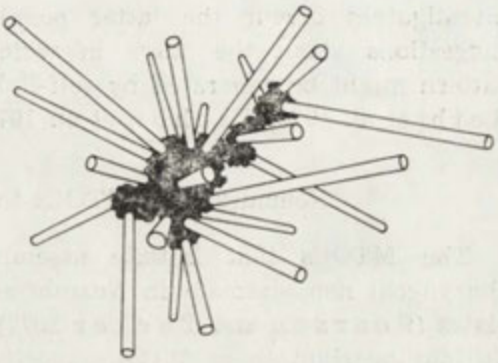

Fig. 3. Schematic diagram showing microtubules growing out in an apparently random variety of directions from an axonemal MTOC in *Echinosphaerium* 7 min after the termination of cold treatment

ample, the two microtubules at the centre of the double-spiral bear more links than those elsewhere in the pattern. Microtubules positioned at the 12 sector boundaries bear one more link than microtubules situated within sectors (Fig. 4). Thus, there are at least 3 'types' of micro-

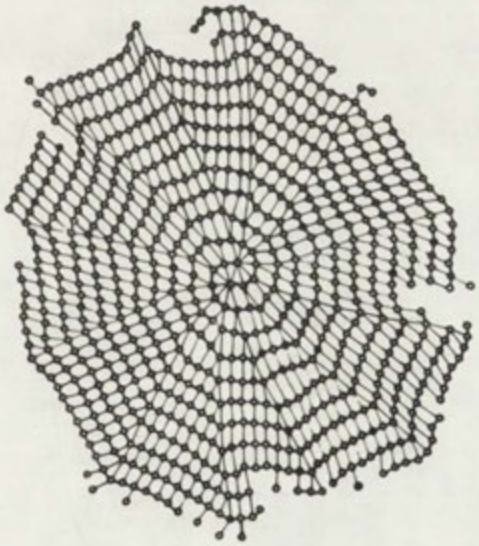

Fig. 4. Cross-sectional profile of an axoneme in *Echinosphaerium* showing the twelve-sectored double-spiral pattern of microtubule packing and linkage. Slightly modifed from MacDonald and Kitching (1967)

tubules. This differentiation might come about because there are 3 species of nucleating elements that initiate 3 different types of microtubules. Alternatively, differentiation may be effected by long-range allosteric interactions between adjacent microtubules that are mediated by links as the pattern is built up, because establishment of pattern apparently always begins at the very centre of the double-spiral. Most

investigators favour the latter possibility. They have made detailed suggestions about the ways in which the 12 sectored double-spiral pattern might be generated by self-linkage (Tilney and Byers 1969, Roth et al. 1970, Cachon et al. 1973, Bardele 1977).

Nemadesmatal MTOCs in the Ciliate *Nassula*

The MTOCs that initiate assembly of the highly-ordered cytopharyngeal nemadesmata in *Nassula* act as microtubule-nucleating-templates (Pearson and Tucker 1977). Unlike the axonemal MTOCs of *Echinosphaerium* these MTOCs specify the spacing and orientation of microtubules with considerable precision.

Nassula possesses a large cytopharyngeal basket that is about 60 μm long. It is employed to manipulate and ingest filaments of blue-green algae. The basket consists mainly of a circular palisade of stiff microtubular nemadesmata. These are joined together by a dense annulus

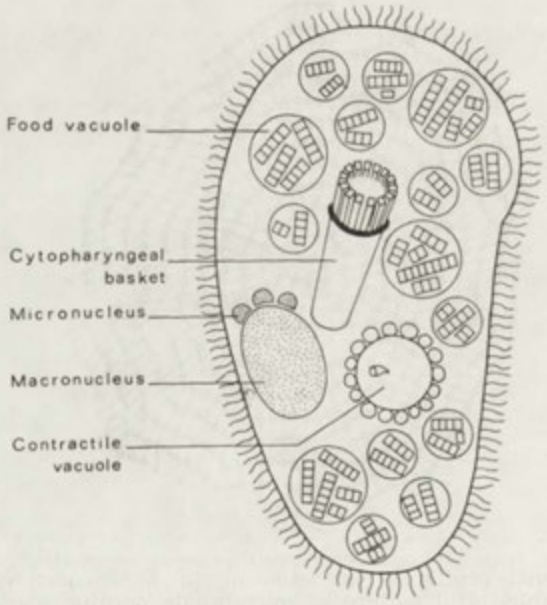

Fig. 5. Schematic diagram of *Nassula* showing the arrangement of its main organelles. The food vacuoles contain fragments of algal filaments

and a microtubular sheath which encircle the basket at certain levels (Fig. 5). During binary fission two new baskets are constructed as the old one disassembles. Each new nemadesmatal microtubule bundle grows out from a laminated sheet that is attached to the proximal end of a basal body (Fig. 6). Microtubules are packed hexagonally and aligned

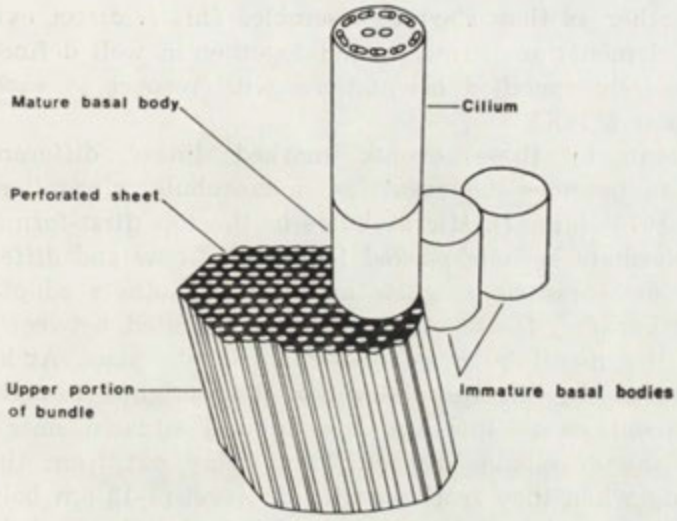

Fig. 6. Schematic diagram showing the association of a growing nemadesmatal microtubule bundle and basal bodies. The uppermost lamina of the sheet of dense material has been omitted to show the hexagonal packing of tubule tops where they penetrate holes in a perforated layer below it. Reproduced from Pearson and Tucker (1977)

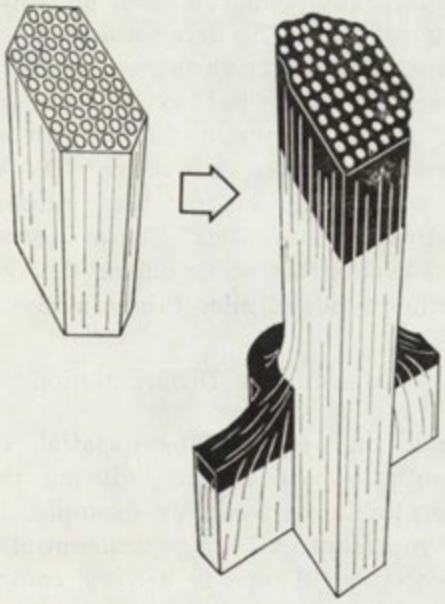

Fig. 7. Schematic diagram showing the arrangement of microtubules and dense intertubular material in a developing nemadesmatal microtubule bundle at two stages in its morphogenesis

with each other as they start to assemble. This is direct evidence that nucleating elements are firmly bound together in well defined positions and at precisely specified orientations with respect to each other in this particular MTOC.

Subsequent to these events marked linear differentiation of nemadesmata becomes apparent as microtubule elongation proceeds (Tucker 1970) (Fig. 7). Microtubules in the top (first-formed) portion of a nemadesmata become packed together in new and different ways. Some take up a rather irregular arrangement, others adopt square or rhomboidal packing. Dense material is concentrated between the upper portions of the microtubules as these changes take place. At lower levels the majority of the microtubules retain their original hexagonal arrangement. Exceptions to this are microtubules situated near the outer surface of the developing basket. They splay out from the sides of nemadesmata when they reach a particular level (5–12 μm below the top of the basket depending on the species of *Nassula*). Some of these microtubules run alongside and inbetween those that have splayed out from adjacent nemadesmata. They form the sheath and are more widely and irregularly spaced than those that retain the hexagonally packed arrangement. They are joined together by links that are longer and thinner than those that connect the hexagonally packed microtubules. Dense material is concentrated between the tops of sheath tubules only at the level where they splay out from the sides of nemadesmata. This band of intertubular material forms the dense annulus.

How are such spatio-temporal variations in microtubule packing and interconnection specified? To what extent can an MTOC contribute spatial instructions for microtubule differentiation at points situated several micrometers away from its surface? Do MTOCs ever include different types of nucleating elements that initiate microtubules with correspondingly distinct potentialities? These questions are pertinent to the interpretation of events that occur during the assembly and differentiation of several other types of microtubule arrays.

Microtubule Differentiation

There is evidence that sophisticated spatial control systems for microtubule differentiation are required during the morphogenesis of many types of microtubule arrays. For example, the tips of suctorian tentacle axonemes represent a very pronounced instance of linear microtubule differentiation. There is a very complex and stringently specified linear sequence of changes in microtubule linkage, number and orientation in this portion of each axoneme (Tucker and Mackie 1975). Basal bodies and centrioles provide examples of very highly-

ordered microtubule arrays that are common to both unicellular and multicellular organisms. The morphogenesis of these organelles is very similar in protozoans, phytoflagellates, metazoan tissue cells and the flagellated gametes of certain lower plants. In all cases microtubules are precisely positioned and oriented when they start to assemble (for example, Dippell 1968). The MTOCs involved act as templates like those which initiate the assembly of nemadesmatal microtubules in *Nassula*. In addition, they specify exactly how many microtubules will assemble — 9 microtubule triplets in most cases. Furthermore, linear differentiation is exhibited. During the formation of cilia and flagella the A and B tubules of the triplets continue to elongate. The C tubules do not. In a basal body the A and B tubules are interconnected in a very different fashion from that which obtains in a ciliary or flagellar axoneme. Hence, in this particular instance, the control of microtubule deployment and differentiation is just as elaborate in multicellular organisms as it is in any of the other situations described so far for protozoans.

Micronuclear spindle microtubules in *Paramecium tetraurelia* provide a striking example of microtubule differentiation. During the final phase of mitotic chromosome separation, the separation spindle is almost entirely composed of microtubules with diameters of 28–32 nm (Tucker 1979). Recent studies in the author's laboratory have established that prior to this, during metaphase and early anaphase when spindle organization is fairly conventional, all the microtubules have diameters of about 24 nm. It is not yet clear if the 24 nm tubules directly differentiate into the larger variety (as a consequence of *in situ* tubulin rearrangement) or if they disassemble and are replaced by a distinct class of larger tubules.

Flagellar microtubules in the amoeboflagellate *Naegleria gruberi* are composed of tubulins that seem to be distinct from those that form microtubules elsewhere in the organism. This discovery provided one of the first really substantial indications that an organism's microtubules are not all identical (Kowit and Fulton 1974). It also led to the 'multi-tubulin hypothesis' which raises the possibility that cells generally possess several types of tubulin monomers, rather than just the two varieties (α and β tubulin) that have been clearly distinguished so far, and that there is a corresponding range of tubulin genes. Hence, microtubules in different organelles, and/or microtubules with different properties in a single microtubule array, might be differentiated because they are composed of different tubulins (Fulton and Simpson 1976). It is not yet clear if the range of tubulins that appear to be available in certain cells is provided because of an equivalent range of tubulin genes, or because a range of post-translatory modifications are

effected on a very much more restricted number of tubulin gene products (Luduena 1979). However, there is evidence that certain genomes include considerably more than two tubulin genes (see Silflow and Rosenbaum 1981).

Since MAPs make a contribution to the fabric of microtubule walls they provide another possible material basis for microtubule differentiation. For example, at least 8 species of MAPs have been distinguished in various cell types to date and it may transpire that the actual number is considerably greater (see Duerr et al. 1981). Hence, the molecular architecture of microtubule walls could be very complex and variable in terms of the range of different proteins that are included in them. Bearing this in mind, it is perhaps not surprising that microtubules can interconnect and interact in a variety of ways at different points along their lengths (possibly in response to local physico-chemical conditions and structural associations that they encounter as they elongate). Nor is it surprising that a cell's microtubules do not all have identical properties. There is the possibility of an equivalent range of different nucleating elements that initiate microtubules which differ in terms of the particular tubulins and MAPs included in their walls. A 'multi-nucleating-element hypothesis' merits further attention.

The notion that the molecular composition of a microtubule wall represents a store of information which defines a microtubule's potential for intertubule linkage and connection to other cell components is valuable in the present context. It may help to explain why the axonemal MTOCs of *Echinosphaerium* apparently contribute very much less extensively to the specification of microtubule positioning (Fig. 3) than the nemadesmatal MTOCs of *Nassula* (Fig. 6). One can speculate that most of the information stored in the walls of nemadesmatal microtubules is required to effect the complex sequence of linear differentiation described above (Fig. 7). As a consequence these microtubules do not 'include' sufficient information to define the initial microtubule arrangement very accurately and the MTOC acts as a template that provides this facility. In marked contrast, the axopodial axonemes of actinophryidian heliozoans do not exhibit well defined linear differentiation. The highly-ordered features of microtubule pattern do not vary appreciably along an axoneme's length. Hence, it can be argued that nearly all the spatial information stored in the walls of these microtubules is devoted to (and is adequate for) establishing the double-spiral pattern by a self-linkage procedure. Thus the MTOC does not act as a template that defines microtubule positioning very precisely. The 'MTOC-template-generation' of basal bodies (Tucker 1977) for linearly differentiated cilia and flagella is compatible with these ideas.

MTOCs in Protozoans and Tissue Cells — Some Important Common Issues

It has been shown that spatial instructions concerning cytoskeletal deployment are sometimes cytoplasmically inherited in certain metazoan cells in tissue culture (Albrecht-Buehler 1977, Solomon 1979). Pericentriolar MTOCs are possible candidates for the cytoplasmic conveyance of such instructions (Solomon 1980 a). There are indications that pericentriolar MTOCs may help to specify the numbers and lengths of the microtubules that grow out towards the surfaces of cells (see Solomon 1980 b). Perhaps, like some of the basal body-associated MTOCs in protozoans (Fig. 6), they also define the initial directions in which particular microtubules will project from their surfaces. Such specifications might ensure that sister cells are sometimes 'identical twins' or 'mirror images' in terms of cell shaping (Albrecht-Buehler 1977, Solomon 1979). Furthermore, it has been suggested that cytoplasmic inheritance of specialised cortical zones may provide a basis for specification of the positions of sub-plasmalemmal microtubule arrays in certain plant tissue cells (Gunning et al. 1978).

All these recently presented ideas and findings for the tissue cells of multicellular organisms are bound to elicit a sense of 'déjà-vu' in certain protozoological circles. The extent to which it has been established that certain protozoan MTOCs control microtubule number, orientation, spacing, and polarity has been reviewed above. Integration of control systems that relate MTOC positioning, cell shaping, and the sizes of microtubule arrays in ciliates has also been scrutinized (Lynn 1977, Golińska and Kink 1977, Bąkowska and Jerka-Dziadosz 1980). Since the unequivocable demonstration of the cytoplasmic inheritance of spatial instructions for cortical basal body pattern in *Paramecium* (Beisson and Sonneborn 1965), investigations of the control of MTOC inheritance and organizing capabilities in protozoans have made substantial progress (see Nanney 1968, Sonneborn 1970, Bardele 1977, Aufderheide et al. 1980). So has analysis of MTOCs and microtubule arrays that are included in mirror image patterns in certain 'doublet ciliates' (Jerka-Dziadosz and Frankel 1979, Grimes et al. 1980). It is quite clear that protozoans often readily reveal phenomena that are basic to eukaryotic cell organization. It is unfortunate that to begin with many of these phenomena and their general importance are frequently dismissed on the grounds that they are peculiar to Protozoa.

REFERENCES

Albrecht-Buehler G. 1977: Daughter 3T3 cells. Are they mirror images of each other? J. Cell Biol., 72, 595–603.

Amos L. A. 1979: Structure of microtubules. In: Microtubules (ed. K. Roberts and J. S. Hyams), pp. 1–64. London, Academic Press.

Aufderheide K. J., Frankel J. and Williams N. E. 1980: Formation and positioning of surface-related structures in *Protozoa*. Microbiol. Rev., 44, 252–302.

Bąkowska J. and Jerka-Dziadosz M. 1980: Ultrastructural aspect of size dependent regulation of surface pattern of complex ciliary organelle in a protozoan ciliate. J. Embryol. exp. Morphol., 59, 355–375.

Bardele C. F. 1977: Comparative study of axopodial microtubule patterns and possible mechanisms of pattern control in the centrohelidian Heliozoa *Acanthocystis*, *Raphidiophrys* and *Heterophrys*. J. Cell Sci., 25, 205–232.

Beisson J. and Sonneborn T. M. 1965: Cytoplasmic inheritance of the organization of the cell cortex in *Paramecium aurelia*. Proc. natl. Acad. Sci. U.S.A., 53, 275–282.

Cachon J., Cachon M., Febvre-Chevalier C. and Febvre J. 1973: Déterminisme de l'edification des systèmes microtubulaire stéréoplasmiques d'Actinopodes. Arch. Protistenk., 115, 137–153.

Dippell R. V. 1968: The development of basal bodies in *Paramecium*. Proc. natl. Acad. Sci. U.S.A., 61, 461–468.

Duerr A., Pallas D. and Solomon F. 1981: Molecular analysis of cytoplasmic microtubules *in situ*: identification of both widespread and specific proteins. Cell, 24, 203–211.

Euteneuer U. and McIntosh J. R. 1981: Polarity of some motility-related microtubules. Proc. natl. Acad. Sci. U.S.A., 78, 372–376.

Fulton C. and Simpson P. A. 1976: Selective synthesis and utilization of flagellar tubulin. The multi-tubulin hypothesis. In: Cell Motility (ed. R. Goldman, T. Pollard and J. Rosenbaum), pp. 987–1005. Cold Spring Harbor Laboratory Publications.

Golińska K. and Kink J. 1977: Proportional regulation of body form and cortical organelle pattern in the ciliate *Dileptus*. J. Cell Sci., 24, 11–30.

Grimes G. W., McKenna M. E., Goldsmith-Spoegler C. M. and Knaupp E. A. 1980: Patterning and assembly of ciliature are independent processes in hypotrich ciliates. Science, N. Y., 309, 281–283.

Gunning B. E. S., Hardham A. R. and Hughes J. E. 1978: Evidence for initiation of microtubules in discrete regions of the cell cortex in *Azolla* root-tip cells, and an hypothesis on the development of cortical arrays of microtubules. Planta, 143, 161–179.

Jerka-Dziadosz M. and Frankel J. 1979: A mutant of *Tetrahymena thermophila* with a partial mirror-image duplication of cell surface pattern. I. Analysis of the phenotype. J. Embryol. exp. Morphol., 49, 167–202.

Jones J. C. R. and Tucker J. B. 1981: Microtubule-organizing centres and assembly of the double-spiral microtubule pattern in certain heliozoan axonemes. J. Cell Sci., 50, 259–280.

Kowit J. D. and Fulton C. 1974: Programmed synthesis of tubulin for the flagella that develop during cell differentiation in *Naegleria gruberi*. Proc. natl. Acad. Sci. U.S.A., 71, 2877–2881.

Luduena R. F. 1979: Biochemistry of tubulin. In Microtubules (ed. K. Roberts and J. S. Hyams), pp. 65–116. London, Academic Press.

Lynn D. H. 1977: Proportional control of organelle position by a mechanism which similarly monitors cell size of wild type and conical form-mutant *Tetrahymena*. J. Embryol. exp. Morphol., 42, 261–274.

MacDonald A. C. and Kitching J. A. 1967: Axopodial filaments of *Heliozoa*. Nature, Lond., 215, 99–100.

Nanney D. L. 1968: Cortical patterns in cellular morphogenesis. Science, N. Y. 160, 496–502.

Pearson P. J. and Tucker J. B. 1977. Control of shape and pattern during the assembly of a large microtubule bundle. Evidence for a microtubule-nucleating-template. Cell Tissue Res., 180, 241–252.

Pickett-Heaps J. D. 1969: The evolution of the mitotic apparatus: an attempt at comparative ultrastructural cytology in dividing plant cells. Cytobios, 3, 257–280.

Roth L. E., Pihlaja D. J. and Shigenaka Y. 1970: Microtubules in the heliozoan axopodium. I. The gradion hypothesis of allosterism in structural proteins. J. Ultrastruct. Res., 30, 7–37.

Silflow C. D. and Rosenbaum J. L. 1981: Multiple α- and β- tubulin genes in *Chlamydomonas* and regulation of tubulin mRNA levels after deflagellation. Cell, 24, 81–88.

Solomon F. 1979: Detailed neurite morphologies of sister neuroblastoma cells are related. Cell, 16, 165–169.

Solomon F. 1980 a: Neuroblastoma cells recapitulate their detailed neurite morphologies after reversible microtubule disassembly. Cell, 21, 333–338.

Solomon F. 1980 b: Organizing microtubules in the cytoplasm. Cell, 22, 331–332.

Sonneborn T. M. 1970: Gene action in development. Proc. R. Soc. Lond. B., 176, 347–366.

Tilney L. G. and Byers B. 1969: Studies on the microtubules in *Heliozoa*. V. Factors controlling the organization of microtubules in the axonemal pattern in *Echinosphaerium* (*Actinosphaerium*) *nucleofilum*. J. Cell Biol., 43, 148–165.

Tucker J. B. 1970: Morphogenesis of a large microtubular organelle and its association with basal bodies in the ciliate *Nassula*. J. Cell Sci., 6, 385–429.

Tucker J. B. 1977: Shape and pattern specification during microtubule bundle assembly. Nature, Lond., 166, 22–26.

Tucker J. B. 1979: Spatial organization of microtubules. In Microtubules (ed. K. Roberts and J. S. Hyams), pp. 315–357. London, Academic Press.

Tucker J. B. and Mackie J. B. 1975: Configurational changes in helical microtubule frameworks in feeding tentacles of the suctorian *Tokophrya*. Tissue and Cell, 7, 601–612.

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Invited Lecture

Environmental Adaptation of *Tetrahymena* Membranes: Significance of Membrane Lipids

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I. Introduction

Since the pioneering work of Marr and Ingraham (1962) has revealed that *E. coli* can survive the unusual cold growth temperature by changing the fatty acid composition, a wealth of information has been available concerning the adaptive response of membrane lipids to various environmental changes in a wide variety of organisms.

Based on the concept that the biological membrane is in a dynamic state which is important for many physiological functions taking place on the membrane, this compositional adaptation serves to maintain a constant membrane fluidity at the temperature of growth, which is known as homeoviscous adaptation process. In general, the decrease in the environmental temperature results in a higher unsaturation of phospholipid fatty acids. However, the molecular mechanism underlying is not well understood.

We have previously demonstrated that a unicellular free-living eukaryote, *Tetrahymena pyriformis* exhibited the marked alterations in membrane lipid composition in response to changes in growth conditions (Thompson and Nozawa 1979, Nozawa and Thompson 1979). Especially, like other organisms, this cell was also shown to undergo considerable modification of membrane lipids during temperature acclimation. Therefore, *Tetrahymena* with defined membrane organ-

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ization is considered to be a potentially promising model system for the better understanding of the adaptive control mechanisms.

This article is intended to review some aspects of membrane lipid modifications observed under the altered growth conditions, and it will also discuss the molecular mechanisms for the thermal lipid adaptation.

II. Adaptive Modification of Membrane Lipids in Response to Environmental Changes

There are many environmental factors which affect the membrane lipid composition of *Tetrahymena* (Nozawa 1980).

(A) Growth Medium

(1) Starvation: Upon shift to the non-nutrient medium (5 mM Phosphate), there is a progressive decrease in cell size to almost half after 24 h-starvation with a large reduction in total phospholipid content. The striking modification occurs during starvation in relative phospholipid and fatty acid compositions (Nozawa et al. 1980 a). As can be

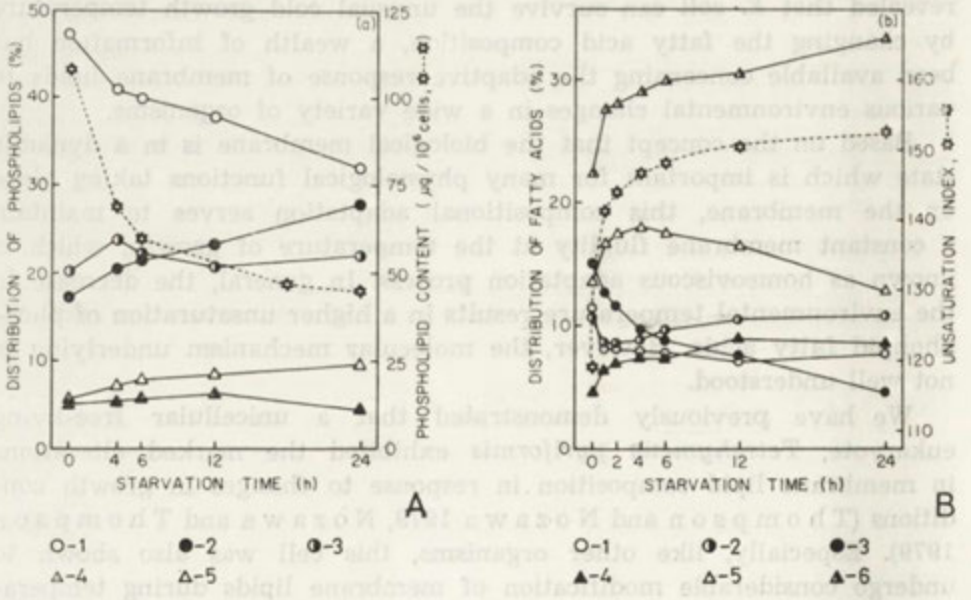


Fig. 1. Alterations in phospholipid and fatty acid composition during starvation in *Tetrahymena*, A — phospholipid composition, 1 — phosphatidylethanolamines, 2 — 2-aminoethylphosphonolipids, 3 — phosphatidylcholines, 4 — cardiolipin, 5 — unknown lipids, B — fatty acid composition, 1 — 14:0, 2 — $16{:}1^{\Delta 9}$, 3 — 16:0, 4 — $18{:}1^{\Delta 9}$, 5 — $18{:}2^{\Delta 9,12}$, 6 — $18{:}3^{\Delta 6,9,12}$

seen in Fig. 1 a, the level of phosphatidylethanolamine (PE) is decreased considerably with a corresponding increase in 2-aminoethylphosphonolipid (AEPL). But the phosphatidylcholine (PC) content remains unchanged. This marked decrease in PE solely present in diacyl-type is due to its higher susceptibility to lipolytic attack by phospholipases as compared with alkyl-types of PC and AEPL containing hexadecyl group at C-1 position. The diacyl-PC shows a great decline but diacyl-AEPL does not. Figure 1 b displays the starvation-induced alterations in the relative proportion of fatty acids. γ-Linolenate (C18:3), the most abundant fatty acid, increases remarkably with a compensatory decrease in palmitoleate (C16:1). However, following transferring the starved cells into an enriched growth medium, such altered lipid composition is rapidly restored to the initial profile of the control cells prior to starvation. From the positional distribution of fatty acids in various phospholipids, it is postulated that hexadecyl/linoleate-PC, palmitate/linoleate-AEPL and hexadecyl/γ-linolenate-AEPL would play crucial roles is adaptation to the nutrient-free environment.

(2) Lipids or its precursors: Some lipids such as sterols (ergosterol, cholesterol, stigmasterol, lanosterol), fatty acids (palmitate, palmitoleate, linoleate, γ-linolenate), sphingomyelin and lipid precursors (isovalerate, choline analogs, hexadecyl glychol) are uptaken by cells to be incorporated into membrane lipids. Accordingly, qualitative or quantitative lipid modifications are to various extents induced in different membranes (Nozawa 1980). For example, cells grown in presence of ergosterol contain tetrahymanol no longer which is instead replaced by added ergosterol. The ergosterol-replacement causes changes in polar head group composition (PE increase and PC decrease) in pellicles and microsomes, and also a decrease both in the average fatty acyl chain length and the relative proportion of unsaturated acids (Nozawa et al. 1975; Pieringer and Conner 1979). Addition of linoleate results in a great increase in this fatty acid in membrane phospholipids, which is concurrently accompanied by a compensating decrease in oleate in pellicles and microsomes, and in linolenate in ciliary membranes (Kasai et al. 1976). Little alteration occurs in the phospholipid class composition. When cells are incubated with choline analogs (ethanolamine, monomethylethanolamine (MME), dimethylethanolamine or choline), only MME is incorporated into membranes as phosphatidyl-MME (Nozawa and Kasai, unpublished). Sphingomyelin can be assembled as intact molecules into different membranes (Ohki et al. unpublished). A novel, as yet unclassified *Tetrahymena* sp. shows an unusually high level of iso-odd-numbered fatty acids, i-C15:0, i-C17:1 which are minor components in classical strains (WH-14, GL, W, NT-1) (Fukushima et al. 1978). In cells incubated in the medium supple-

mented with 2 mM isovalerate, almost 60% of the total membrane fatty acid is comprised of these two iso fatty acids.

(3) Cations and drugs: *Tetrahymena* can grow in high concentration of cations (Mattox and Thompson 1980). The phospholipid composition of pellicles from cells fully acclimated to high salinity (0.3 M NaCl) shows a rise of PE (39→48%), for which decreases in PC and AEPL are compensating. There is no appreciable change in fatty acid composition in Na^+-acclimated cells. Cells transferred from the normal medium to 0.1 M Ca^{2+} medium resume growth after a lag of 1–2 h. Unlike the case of high-Na^+ adaptation, acclimation to high Ca^{2+} is not accompanied by any significant alteration in membrane lipid composition.

When *Tetrahymena* cells are exposed to drugs (phenethyl alcohol, ethanol, lipid metabolism inhibitors, anesthetics), there are changes with various degrees in lipid compositions (Nozawa 1980). The membranes from cells incubated with phenethyl alcohol contain a higher level of PC with a compensatory decrease in PE, while AEPL shows a small decrease (Nozawa et al. 1979). The fatty acid composition in membrane phospholipids is also modified with a profound elevation of the content of linoleate and linolenate and a decrease in palmitoleate. Such lipid alteration is reversible, and upon removal of the drug the profile of modified lipid composition returns to normal. When cells are grown in a medium containing high concentration (1.6%) of ethanol, its membrane lipid composition is significantly different from that of control cells (Nandini-Kishore et al. 1979). The principal changes include a decline by 18% in palmitoleate and a corresponding increase in linoleate, and also a large reduction of AEPL and a concurrent rise in PE. This ethanol-induced lipid modification reverts to normal quickly after the transfer to normal medium. The antibiotic cerulenin, a potent inhibitor of the condensing enzyme in the fatty acid synthetase complex, causes some significant changes in lipid composition; an increase in PE and a decrease in palmitoleate (Nozawa, unpublished). Triparanol inhibits the squalene cyclization, thereby resulting in a reduction of tetrahymanol content in pellicles. Although little or no change is observed in phospholipid composition, a small but significant decrease in palmitoleate and an increase in γ-linolenate is observed.

(4) Aging: The culture medium in the stationary phase is nutrientless and insufficient for active cell growth. The membrane lipids of *Tetrahymena* are altered in a manner markedly dependent on the progress of growth age (Nozawa et al. 1980 b). The general trend is a considerable decrease in content of PE accompanied by a small increase in PC. As for fatty acid composition, the most notable change occurs in unsaturated fatty acids; a great rise in oleate and linoleate

with a compensatory decrease in palmitoleate. This leads to an augmented unsaturation of the overall phospholipid fatty acid profile of the aged membranes.

(B) Temperature Acclimation

(1) Isothermal growth at different temperatures: Like diverse microorganisms, *Tetrahymena* cells strive to survive at either suboptimal or supraoptimal temperatures by adaptive modification of membrane lipids in such a way as to maintain the proper fluidity (Fukushima et al. 1976). A thermotolerant strain NT-1 has been proved to be a greatly useful system for studying the thermal adaptation at the molecular level, because whereas other strains have the upper limit for growth at around 35°C, NT-1 cells are capable of active growth even at 41°C. When cells are grown at different temperatures (15°C, 24°C, 39.5°C), their lipid compositions are modified. There is a decrease in AEPL and a corresponding increase in PE as the growth temperature increases, e.g., 26% (15°C), 34% (24°C) and 43% (39.5°C). The level of phospholipids with glyceryl ether (hexadecyl/acyl-PC, hexadecyl/acyl-AEPL) is enhanced considerably with reducing temperature. There is little or if any small change in the tetrahymanol content. The principal effects on fatty acid composition of decreasing temperature are an increase in linoleate and linolenate and a decrease in palmitate. These lipid alterations of whole cells are reflected in all membrane fractions in an identical but rather augmented manner. The ciliary membrane shows a relatively small change as compared with pellicles and microsomes.

The positional distribution of the fatty acyl chains in the major phospholipids is compared between 15°C- and 39.5°C-grown cells. As for PE at the C-1 position the most abundant fatty acid at 39.5°C is palmitate and at 15°C is γ-linolenate, while at the C-2 position the richest fatty acid is palmitoleate at 39.5°C and at 15°C is linoleate (Watanabe et al. 1979). There is a similarity in fatty acid composition between diacyl-PC and diacyl-AEPL in 39.5°C- and 15°C-grown cells (Watanabe et al. 1980). However, marked increases in γ-linolenate at C-1 position in diacyl-PC and in linoleate at C-2 position of diacyl-AEPL are observed in 15°grown cells. In contrast, the C-2 positions of alkyl type -PC and -AEPL are occupied by polyunsaturated fatty acids. In the cold cells, the sum of linoleate and γ-linolenate accounts for 71% in hexadecyl/acyl-PC and 53% in hexadecyl/acyl-AEPL, which contains a high level (40%) of an unusual fatty acid, cilienic acid ($C18:2^{\Delta 6,11}$).

(2) Temperature-shift: This organism has an ability to acclimate to rapid growth temperature change (shift-down or-up) (Wun-

Table 1

Alterations in fatty acid composition of various membrane fractions from *Tetrahymena pyriformis* NT-1 cells during temperature acclimation

Fatty Acid	Mitochondria					Pellicles					Microsomes				
	Time after shift to 15°C					Time after shift to 15°C					Time after shift to 15°C				
	(39°C)	2h	4h	6h	10h	(39°C)	2h	4h	6h	10h	(39°C)	2h	4h	6h	10h
14 : 0	5.8	6.0	5.8	5.0	4.8	8.7	8.6	8.6	7.9	8.5	7.9	8.8	7.9	7.4	7.2
16 : 0	13.2	11.1	10.1	7.4	6.7	18.4	15.6	13.4	10.8	11.0	15.4	12.6	10.9	8.4	8.1
16 : 1	10.2	11.1	11.5	11.4	10.2	9.9	11.8	13.2	13.3	12.0	11.2	13.5	14.5	14.3	12.9
16 : 2*	4.5	4.8	4.6	5.2	4.9	4.3	4.8	5.1	5.9	5.1	5.4	5.8	5.9	6.7	6.2
18 : 1	7.9	6.8	6.8	6.3	6.1	10.6	8.5	7.4	7.0	7.4	10.1	6.4	6.6	6.0	6.5
18 : 2	18.1	18.1	17.9	17.4	18.6	13.5	14.7	16.3	15.6	16.3	14.5	14.8	16.8	15.1	16.7
18 : 3	28.1	28.3	29.5	32.7	35.0	18.7	20.0	20.8	23.5	24.1	20.9	22.2	23.4	26.3	27.9
U/S**	2.65	2.73	3.04	3.86	4.36	1.45	1.65	1.82	2.12	2.10	1.80	1.86	2.22	2.55	2.78

* Contains also small amounts of 17 : 0 which was not separable from 16 : 2 under conditions used.
** A ratio of unsaturated to saturated fatty acids.

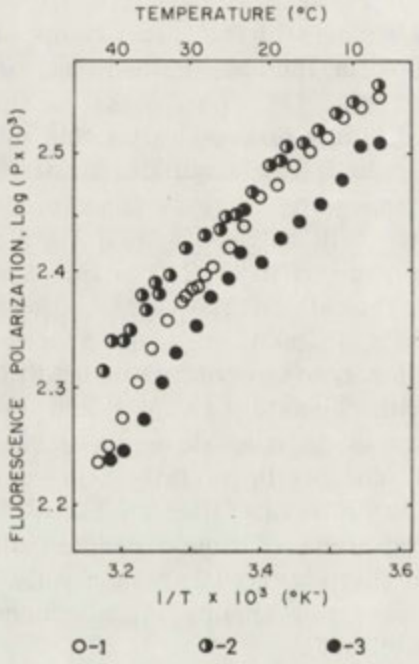
derlich et al. 1973; Fukushima et al. 1976). Following the shift from 39.5°C to 15°C over a 30-min period, occur changes in phospholipid, as well as fatty acyl chain, compositions (Fukushima et al. 1976). Neither cell division nor alteration in phospholipid class composition occurs within 10 h after the temperature shift. But a noticeable change is seen in the relative proportion of individual phospholipids, thereafter, and AEPL increases drastically at the expense of PE (Nozawa and Kasai 1978). The level of PC is rather constant. By contrast, a rapid response to temperature changes of fatty acid composition is induced. Palmitoleate (C16:1) begins to increase immediately after the shift and reaches the maximal level at 4 h, whereas the palmitate (C16:0) content is concurrently reduced (quick process). After this short-term adaptation process, the level of palmitoleate continues to decrease and instead γ-linolenate shows a corresponding increase (slow process). It takes nearly two days for the shifted cells to restore their fatty acid composition to that of cells isothermally grown at 15°C. The increases in γ-linolenate at the C-1 position and linoleate at the C-2 position are commonly observed in diacyl-types of PE, PC and AEPL) (Watanabe et al. 1980, 1981). The C-2 position of alkyl-types of PC and AEPL is occupied mainly by γ-C18:3 together with cilienate (C18:2$^{\Delta 6,11}$) and γ-linoleate (C18:2$\triangle^{9,12}$). There is a small increase in cilienate and γ-linolenate only in alkyl-type PC after the shift. The profile of fatty acid composition of membrane fractions at various intervals after the shift-down is summarized in Table 1. The general trend of time-dependent alterations is similar or almost identical to that observed in whole cells, except that the level of palmitoleate does not change in mitochondria (Maruyama and Nozawa, unpublished).

III. Effects of Lipid Modification on Membrane Fluidity

According to the widely accepted concept that lipid composition exerts much influence on membrane fluidity, it seem feasibly to expect that various adaptive modifications of membrane lipid composition as described above should affect the physical properties of *Tetrahymena* membranes.

By ESR analysis, cilia, pellicles and microsomes isolated from cells grown in the presence of ergosterol show lower fluidities than membranes from control cells containing tetrahymanol. This rigidifying effect of ergosterol is also observed as an altered distribution pattern of membrane particles by freeze-fracture electron microscopy (Nozawa 1980). On the other hand, membranes from hexadecyl glycerol-supple-

mented cells are in a more fluid state as compared with control membranes (Fukushima et al. 1976). These changes in membrane fluidity are confirmed by fluorescence polarization using diphenyl hexatriene (Shimonaka et al. 1978). Figure 2 displays an example of pellicles.



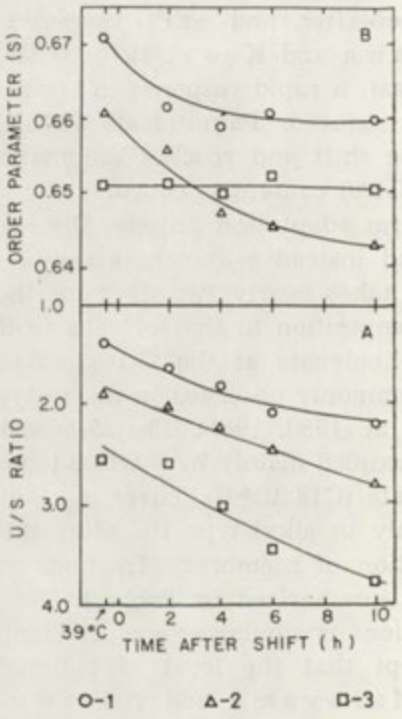


Fig. 2. Alterations in fluidity of lipid-modified pellicles of *Tetrahymena*, 1 — control, 2 — ergosterol-fed, 3 — hexadecyl glycerol-fed

Fig. 3. Alterations in fluidity and U/S ratio of various membranes during cold acclimation in *Tetrahymena*, 1 — pellicles, 2 — microsomes, 3 — mitochondria

The membranes of cilia, pellicles and microsomes from cells grown isothermally at 15°C have greater fluidities than those from cells grown at 39.5°C (Iida et al. 1978). During the acclimation period after shift from 39.5°C to 15°C, a gradual increase in fluidity is seen in cilia, pellicles and microsomes but not in mitochondria (Fig. 3), (Yamauuhi et al. 1981). The possible mechanism for this consistence of fluidity of mitochondrial lipids will be discussed in the next section. In addition, the physical state of microsomal lipids has extensively been examined by other investigators for either cells grown isothermally at different temperature of acclimating cells after shift-down (Wunderlich et al. 1973, 1975, 1978, Martin and Thompson 1978, Dickens and Thompson 1980).

When cells are grown in a culture medium supplemented with linoleate, their membranes contain a high concentration of this fatty acid at the expense of oleate. The distribution pattern of membrane particles as examined by freeze-fracture electron microscopy suggest that membranes of linoleate-supplemented cells are more fluid than those of control cells (Martin et al. 1976).

The pelliclar membrane of phenethyl alcohol-treated cells reveals less aggregation of membrane particles, as compared with control membranes. The quantitative analysis of the thermotropic lateral movement of membrane indicates that the membranes of phenethyl alcohol-supplemented cells become more fluid (Nozawa et al. 1979). This may result from an increase in acyl group unsaturation.

The ESR spectra of spin-labeled phospholipids extracted from pellicles and microsomes lead to the suggestion that these membranes have greater fluidities.

IV. Molecular Mechanisms for Thermal Adaptation of Membrane Lipids

Although much information has been accumulating about adaptive modifications of phospholipid fatty acyl chains in diverse organisms, the underlying mechanisms are not well understood. The molecular mechanism causing considerable enhancement of unsaturation in *Tetrahymena* membrane phospholipids seem to be more complex than expected. Here some possible processes are discussed with regard to thermal lipid adaptation in *Tetrahymena*.

(A) Regulation of Desaturase Activity

As shown in Fig. 3 and Table 1, the cold acclimation is associated with a profound increase in fatty acid unsaturation in membrane phospholipids, in which desaturases are implicated. Hence it follows that cells acclimated to the cold temperature have enhanced desaturase activities.

When cells are pulse-labeled with ^{14}C-palmitate at intervals after shift-down to 15°C, the produced ^{14}C-palmitoleate content rises immediately after shift, reaching the maximal level at 2 h thereafter (Nozawa and Kasai 1978). This maximum of the conversion rate from ^{14}C16:0 to ^{14}C16:1 is well compatible with that of palmitoyl-CoA desaturase activity. At present, two regulatory mechanisms of the desaturase are proposed, and somehow related with membrane fluidity. One is an increase in the amount of desaturases (induction) and the other is an increase in the activity of these enzymes (direct fluidity control).

(1) Induction: As mentioned above, the maximal peak of palmitoyl-CoA desaturase appears at 2 h after shift (Fig. 4). However, cells exposed to either cycloheximide, puromycin or actinomysin D show remarkable repression of this enzyme activity, and the sharp peak observed with control cells is no longer distinct (Nozawa and Kasai, unpublished).

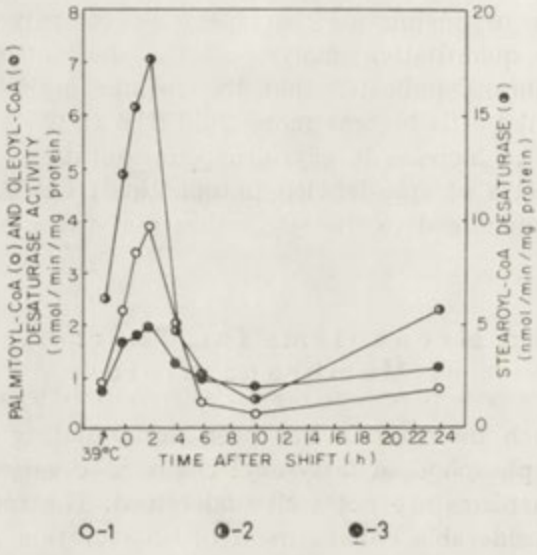


Fig. 4. Enhancement of desaturase activities after temperature shift to 15°C in *Tetrahymena*, 1 — palmitoyl-CoA desaturase, 2 — stearoyl-CoA desaturase, 3 — oleoyl-CoA desaturase

Similar findings are also obtained in the starved cells which are deprived of pre-existing desaturase, demonstrating a geat enhancement of desaturase activity and its marked inhibition by cycloheximide (Kasai and Nozawa 1980). This provides evidence to support the concept of induction, because the starved cells having little or no significant desaturase activity show a temperature-induced rise in the enzyme activity to the level of control shifted cells, probably resulting from reutilization of degraded cell components. At present, the exact sites of enzyme synthesis, in which membrane fluidity is involved, are not yet determined. The activities of stearoyl-CoA and oleoyl-CoA desaturases involving increase in polyunsaturated fatty acids are also thought to be enhanced by the same mechanism as that for palmitoyl-CoA desaturase (Fukushima and Nozawa, unpublished).

(2) Direct fluidity control: According to this hypothesis, the activity of pre-existing desaturase molecules is regulated not by a direct effect of temperature on the enzyme, but by the fluidity of microsomal membrane in which the desaturases are situated (Skriver

and Thompson 1979, Thompson 1980). When membrane fluidity can be modified isothermally by several procedures as described above, the activity of fatty acid desaturation is altered depending on fluidity changes (Martin et al. 1976). Decreased fluidity is accompanied by increased desaturation activity and vice versa. A plausible regulatory mechanism of desaturase is proposed, suggesting a perpendicular movement of the enzyme molecules which facilitates accesibility of fatty acid substrate.

The other likely candidate is phosphorylation-dephosphorylation system as observed with HMG-CoA reductase. But there is no available information to support this hypothesis.

(B) Renewal of Specific Phospholipid Molecular Species

Many experiments have indicated that not only the overall composition but also the positional distribution of fatty acids in phospholipids affects greatly membrane fluidity. The acclimating cells undergo marked rearrangement of fatty acyl chains in phospholipids. In PE which is the most abundant constituent in all membrane fractions and is present solely in diacyltype, a progressive increase in linoleate and γ-linolenate is observed in the overall composition, with the corresponding decrease in palmitate. Palmitoleate, which is responsible for the quick adaptation process, reaches a maximal level after 6 h-incubation at 15°C. It is of particular importance to note that palmitate at the C-1 position might be replaced with γ-linolenate, since such substitution of saturated fatty acid with polyunsaturated fatty acid is feasibly expected to exert a drastic fluidizing effect. Therefore, the renewal of a specific molecular species, γ-linolenate/linoleate (or palmitoleate) would play a crucial role in the efficient thermal adaptation in *Tetrahymena*. Table 2 shows the positional distribution of fatty acids in PE from mitochondria and microsomes (Maruyama, Banno and Nozawa, unpublished). Although a rise in γ-linolenate at C-1 position is considerable in microsomes, it is much less marked in mitochondria containing the high basal level of this fatty acid. As for the C-2 position, there is the common trend of increases in palmitoleate and linoleate and a decrease in oleate in both membrane fractions, with its extent being more pronounced in microsomes than mitochondria. Surprisingly, the relative proportion of γ-linolenate at C-2 position is low and does not show little change after the temperature shift.

(C) Role of Cardiolipin in Mitochondrial Membrane Adaptation

Following the growth temperature-shift from 39.5°C to 15°C, membranes of cilia, pellicles and microsomes increase fluidity with time. However, mitochondrial membrane is exceptional and causes no change

Table 2

Alterations in positional distribution of fatty acids in phosphatidylethanolamine of mitochondria and microsomes from *Tetrahymena pyriformis* NT-1 cells during temperature acclimation

Fatty acid	C-1 position				C-2 position			
	Time after shift to 15°C				Time after shift to 15°C			
	39°C	2h	6h	10h	39°C	2h	6h	10h
Mitochondria								
14 : 0	11.0	10.5	15.4	13.5	3.5	2.8	3.3	4.3
iso 15 : 0	7.8	6.8	9.9	8.2	1.3	0.8	0.9	1.0
16 : 0	26.5	22.6	22.0	15.3	3.5	2.2	2.5	3.7
16 : 1	7.5	7.6	9.6	10.6	16.6	21.3	25.1	23.0
16 : 2*	2.7	3.3	3.8	5.3	7.2	8.8	9.9	9.3
18 : 1	1.1	1.4	1.0	1.8	24.6	16.6	17.2	14.4
18 : 2	2.1	3.2	1.6	3.4	18.1	21.6	18.2	20.4
18 : 3	31.0	36.4	26.0	29.5	11.6	13.3	9.4	10.9
Microsomes								
14 : 0	17.3	20.5	16.8	13.5	4.1	2.7	2.5	2.5
iso 15 : 0	12.2	13.2	11.1	9.0	1.3	0.8	0.6	0.8
16 : 0	34.7	28.7	19.7	15.6	3.1	3.0	2.1	1.9
16 : 1	9.6	9.4	12.5	11.1	16.4	26.7	31.2	23.6
16 : 2*	3.1	3.3	5.0	6.0	7.8	10.5	12.7	11.0
18 : 1	1.1	0.6	1.6	1.1	29.0	13.7	14.0	14.5
18 : 2	2.4	2.3	2.7	2.9	13.6	17.9	16.7	22.1
18 : 3	8.5	11.1	17.9	25.2	7.1	10.1	7.8	11.1

* Contains also small amounts of 17 : 0 which was not separable from 16 : 2 under conditions used.

in fluidity as demonstrated in Fig. 3. There would be several explanations for the mechanisms by which mitochondrial membrane fluidity is maintained at a constant level during the 10 h-acclimation period. Among the major membrane fractions examined, mitochondria have the highest content of unsaturated fatty acids, as indicated by the U/S ratio in Fig. 3. Therefore, unlike other three membranes, the physical properties of mitochondrial membrane may be unaffected by further increase in fatty acid unsaturation. An alternative explanation is involvement of cardiolipin richest in mitochondria. To test this possibility, cardiolipin was removed by thin-layer chromatography from mitochondrial total lipids extracted from 39.5°C-grown cells and 15°C-acclimated cells, and the cardiolipin-depleted mitochondrial lipids were examined by ESR technique. The results indicate that removal of cardiolipin causes no change in order parameter of mitochondrial lipids from 39.5°C-grown cells, whereas it does decrease the parameter of mito-

chondrial lipids from 15°C-acclimated cells (Yamauchi et al. 1981). This implies that cardiolipin would exert a rigidifying effect on acclimated cell's mitochondria. Since no modification occurs in fatty acyl chain composition (linoleate 36%, γ-linolenate 45%) of cardiolipin during the cold acclimation, the lowering effect of mitochondrial fluidity would not be due to cardiolipin's acyl group. More detailed experiments should be required to explore this complex mechanism.

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REFERENCES

Dickens B. F. and Thompson G. A. 1980: Effects of growth of different temperatures on the physical state to lipids in native microsomal membranes from *Tetrahymena*. Biochemistry, 19, 5029–5037.

Fukushima H., Martin C. E., Iida H., Kitajima Y., Thompson G. A. and Nozawa Y. 1976: Changes in membrane lipid composition during temperature adaptation by a thermotolerant strain of *Tetrahymena pyriformis*.

Fukushima H., Watanabe T. and Nozawa Y. 1976: Studies on *Tetrahymena* membranes. In vivo modification of membrane lipids by 1-0-hexadecyl glycerol-feeding in *Tetrahymena pyriformis*. Biochim. Biophys. Acta, 436, 249–259.

Fukushima H., Kasai R., Akimori N. and Nozawa Y. 1978: A novel *Tetrahymena* sp. with unusual membrane lipid composition, with special reference to isofatty acyl chains. Jap. J. Exp. Med., 48, 373–380.

Iida H., Maeda T., Ohki K., Nozawa Y. and Ohnishi S. 1978: Transfer of phosphatidylcholine between different membranes in *Tetrahymena* as studied by spin labeling. Biochim. Biophys. Acta, 508, 55–64.

Kasai R., Kitajima Y., Martin C. E., Nozawa Y., Skriver L. and Thompson G. A. 1976: Molecular control of membrane properties during temperature acclimation. Membrane fluidity regulation of fatty acid desaturase activity. Biochemistry, 15, 5228–5233.

Kasai R. and Nozawa Y. 1980: Regulatory mechanism of palmitoyl-CoA desaturase activity in thermal adaptation. Induction in non-growing *Tetrahymena* cells deprived of pre-existing desaturase. Biochim. Biophys. Acta, 617, 161–166.

Marr A. G. and Ingraham J. L. 1962: Effect of temperature on the composition of fatty acids in *Escherichia coli*. J. Bacteriol., 84, 1260–1269.

Martin C. E., Hiramitsu K., Kitajima Y., Nozawa Y., Skriver L. and Thompson G. A. 1976: Molecular control of membrane properties during temperature acclimation. Fatty acid desaturase regulation of membrane fluidity in acclimating *Tetrahymena* cells. Biochemistry, 15, 5218–5227.

Martin C. E. and Thompson G. A. 1978: Use of fluorescence polarization to monitor intracellular membrane changes during temperature acclimation,

Correlation with lipid compositional and ultrastructural changes. Biochemistry, 17, 3581–3586.

Mattox S. M. and Thompson G. A. 1980: The effects of high concentration of sodium or calcium ions on the lipid composition and properties of *Tetrahymena* membranes. Biochim. Biophys. Acta, 599, 24–31.

Nandini-Kishore S. G., Mattox S. M., Martin C. E. and Thompson G. A. 1979: Membrane changes during growth of *Tetrahymena* in the presence of ethanol. Biochim. Biophys. Acta, 551, 315–327.

Nozawa Y., Fukushima H. and Iida H. 1975: Studies on *Tetrahymena* membranes. Modification of surface membrane lipids by replacement of tetrahymanol by exogenous ergosterol in *Tetrahymena pyriformis*. Biochim. Biophys. Acta, 406, 248–263.

Nozawa Y. and Kasai R. 1978: Mechanism of thermal adaptation of membrane lipids in *Tetrahymena pyriformis* NT–I. Possible evidence for temperature-mediated induction of palmitoyl-CoA desaturase. Biochim. Biophys. Acta, 529, 54–66.

Nozawa Y., Kasai R. and Sekiya T. 1979: Modification of membrane lipids. Phenethyl alcohol-induced alteration of lipid composition in *Tetrahymena* membranes. Biochim. Biophys. Acta, 552, 38–52.

Nozawa Y. 1980: Modification of lipid composition and membrane fluidity. In: Membrane Fluidity: Biophysical Techniques and Cellular Regulation (M. Kates and A. Kuksis ed.), pp. 399–418, Humana Press, New Jersey.

Nozawa Y. and Thompson G. A. 1979: Lipids and membrane organization in *Tetrahymena*. In Biochemistry and Physiology of Protozoa (M. Levandowsky and S. H. Hutner ed.), pp. 276–338, Academic Press, New York.

Nozawa Y., Kasai R. and Sekiya T. 1980a: Modifications of membrane lipid composition following the nutritional shift-up of starved cells. A comparison with membrane biogenesis in *Tetrahymena*. Biochim. Biophys. Acta, 603, 347–365.

Nozawa Y., Kasai R., Kameyama Y. and Ohki K. 1980b: Age-dependent modifications in membrane lipids. Lipid composition, fluidity and palmitoyl-CoA desaturase in *Tetrahymena pyriformis*. Biochim. Biophys. Acta, 599, 232–245.

Pieringer J. and Conner R. L. 1979: Positional distribution of fatty acids in the glycerophospholipids of *Tetrahymena pyriformis*. J. Lipid Res. 20, 363–370.

Shimonaka H., Fukushima H., Kawai K., Nagao S., Okanao Y. and Nozawa Y. 1978: Altered microviscosity of in vivo lipid manipulated membranes in *Tetrahymena pyriformis*: A fluorescence study. Experientia, 34, 586–587.

Skriver L. and Thompson G. A. 1979: Temperature-induced changes in fatty acid unsaturation of *Tetrahymena* membranes do not require induced fatty acid desaturase synthesis. Biochim. Biophys. Acta, 572, 376–381.

Thompson G. A. and Nozawa Y. 1979: A system for studying dynamic membrane alterations within the eukaryotic cell. Biochim. Biophys. Acta (Rev. Biomembrane), 472, 55–92.

Thompson G. A. 1980: Regulation of membrane fluidity during temperature acclimation of *Tetrahymena pyriformis*. In Membrane Fluidity: Biophysical Techniques and Cellular Regulation (M. Kates and A. Kuksis ed.), pp. 381–397.

Watanabe T., Fukushima H. and Nozawa Y. 1979: Studies on temperature adaptation in *Tetrahymena*. Positional distribution of fatty acids and species analysis of phosphatidylethanolamine from *Tetrahymena pyriformis* grown at different temperatures. Biochim. Biophys. Acta, 573, 363–374.

Watanabe T., Fukushima H. and Nozawa Y. 1980: Studies on thermal adaptation in *Tetrahymena pyriformis*. Positional distribution of fatty acids in diacyl- and alkyl-acyl-phosphatidylcholines and -(2-aminoethyl)phosphonolipids from cells grown at different temperatures. Biochim. Biophys. Acta, 62, 133–141.

Watanabe T., Fukushima H., Kasai R. and Nozawa Y. 1981: Studies on thermal adaptation in *Tetrahymena* membrane lipids. Changes in positional distribution of fatty acids in diacyl-phospholipids and alkyl-acyl-

phospholipids during temperature acclimation. Biochim. Biophys. Acta (in press).

Wunderlich F., Speth V., Batz W. and Kleinig H. 1973: Membranes of *Tetrahymena*. III. The effect of temperature on membrane core structures and fatty acid composition of *Tetrahymena* cell. Biochim. Biophys. Acta, 298, 39–49.

Wunderlich F., Ronai A., Speth V., Seelig J. and Blume A. 1975: Thermotropic lipid clustering in *Tetrahymena* membranes, Biochemistry, 17, 3730–3735.

Wunderlich F., Kreutz W., Mahler P., Ronai A. and Heppeler G. 1978: Thermotropic fluid → order "discontinous" phase separation in microsomal lipids of *Tetrahymena*. An X-ray diffraction study. Biochemistry, 17, 2005–2010.

Yamauchi T., Ohki K., Maruyama H. and Nozawa Y. 1981: Thermal adaptation of *Tetrahymena* membranes with special reference to mitochondria. Role of cardiolipin in fluidity regulation of mitochondrial membranes. Biochim. Biophys. Acta (submitted for publication).

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Invited Lecture

Protozoology in Poland — Past and Present

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Foreward

Political history has been an important factor of sciences development in Poland. This has especially been true for the last two centuries, the period in which science acquired its modern shape. In 1772 the armies of Prussia, Austria and Russia invaded the Republic of Poland and occupied a third of the area of the country. In 1793 Prussia and Russia took control of a further third of the Polish territory and in 1795 together with Austria seized the remainder. The Polish State was lost for 123 years. During this period, the Polish nation many times rose up in arms against the occupying powers. The collapse of each successive bid for freedom resulted in an increase in repression against the Polish population, against the Polish culture.

Since 1869 there were no Polish universities, no Polish research institutions in the Russian-occupied and in the Prussian-occupied areas, only in the Austrian-occupied southern Poland there was more freedom for activity of Polish scientists.

For that reason in the 19th and the beginning of the 20th centuries, quite often Poles tried to do scientific research abroad, where they had emigrated or in outlying places where they had been banished. On November 11, 1918 the German occupation forces in Warsaw were disarmed and the Polish State began to rebuild, as parliamentary democracy. The frontiers of Poland were finally fixed in 1922, after the Third Silesian Uprising and war against the Soviet Russia. After 17

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years, on September 1, 1939, German forces invaded Poland and the Second World War began. The last 36 years after the end of this most blooded war is the longest relatively quiet period in Poland, since 1772.

The Founders

Poland's violent history has not helped in the development of science, but in spite of all straightened circumstances Poles made during 19th century and in the 20th considerable contribution to progress many branches of science, including protozoology.

The original study of Protista in Poland and in Russia dated from 1855 when the first papers of Leon Cienkowski[1] (1822–1887) were published.

Fig. 1. Leon Cienkowski (1822–1887)

Leon Cienkowski, working at Russian universities and German and French scientific institutions advanced the whole field of protistology, including the study of flagellates, amoebas, ciliates, myxomycetes, algae and bacteria. Cienkowski (1855) was the first one, who sucessfully criticized F. Stein's "Acineten-theory". Among the protozoological investigations his the earliest studies were made on the structure and encystment processes of ciliates.

His comparative studies on flagellates, ciliates and algae showed in contrary to prevalent opinion, the all the unicellular organisms are

[1] Cienkowski from his birth to graduation from high school in 1838 lived in Warsaw. In this year he passed the entrance examination for the St. Petersburg University and that was for him one and the only chance for university study. After closing of the Royal Warsaw University (1831) by Tsar Nicolaus for 25 years there was no university in the Russian-occupied part of Poland. Cienkowski had never had the opportunity to do scientific researches in his own country. In 1862, a new university was open in Warsaw, named the Principal School. Cienkowski with pleasure admitted the appointment as a professor and chairman of the Department of Anatomy and Cytology of Plants at the Principal School. The January 1863 Uprising, lasting to autumn 1864 prevented Cienkowski's arrival to Warsaw. In 1869 the Principal School was closed down and the Tsar Warsaw University (1870–1914) established with Russian language and the majority of Russian professors.

fundamentally the same and the border between animals and plants does not exist in nature.

In sixties Cienkowski shifted his focus from protozoa and algae to myxomycetes and gave the description of reproduction on life cycle, of these organisms. In this new field his study was also made on motile behaviour of myxomycetes plasmodia. Cienkowski is the author of the term "plasmodium" and he was the first one, who established acellular stage of myxomycetes as one of the most favorable material for the analysis of primitive motile systems. Later Cienkowski turned again to studies of amoebae, flagellates and ciliates.

Cienkowski laid broad and deep foundations for many later works on the biology unicellular organisms and was widely recognized as a pioneering protistological investigator. During his life Cienkowski published articles in Polish on protozoa and, other themes and has had considerable influence on development of protozoological researches in Warsaw, as a teacher and close friend of August Wrześniowski (1836–1892). Wrześniowski met Cienkowski at the St. Petersburg University, where he completed studies on law and later on biological sciences. Back in Warsaw (1861) Wrześniowski quickly was promoted as assistant (1862) and later in 1864 as the professor and chairman of the Department of Zoology and Comparative Anatomy at the Physico-Mathematical Faculty of the Principal School.

Fig. 2. August Wrześniowski (1836–1892)

During six years of activity at the Principal School (1862–1869) and twenty years of professorship as the chairman of the Department of Zoology (1869–1889) of Tsar Warsaw University August Wrześniowski published pioneer papers on morphology, ecology, physiology and systematics of ciliates. He described 86 species of ciliates being found by him in Warsaw and Warsaw region. Between them ten were described as sp. n. In addition to his descriptions, detailed figures were also enclosed.

Majority of species designated and figured by Wrześniowski were accepted in the Bronn's taxonomic monography "Klassen und Ordnungen, Thier-Reiches Ed. I, (1887–1889). In his taxonomic descript-

ions of *Zoothamnium* Cienkowski (1877) included the observation on the response to stimuli and contraction of the stalk.

Wrześniowski described structures relating to the feeding apparatus of different ciliates, food uptake and movement of the food vacuoles. Wrześniowski gained fame not only as protozoologists but also as an author of papers on anatomy and systematics of crustacea and anatomy of *Lamellibranchiata* and numerous articles on evolution and Darwin's theory. This theory Wrześniowski presented to students since 1863, on regular course of zoology.

Wrześniowski was also an excellent teacher and in effect from Wrześniowski's time Warsaw became the main centre of protozoological researches in Poland.

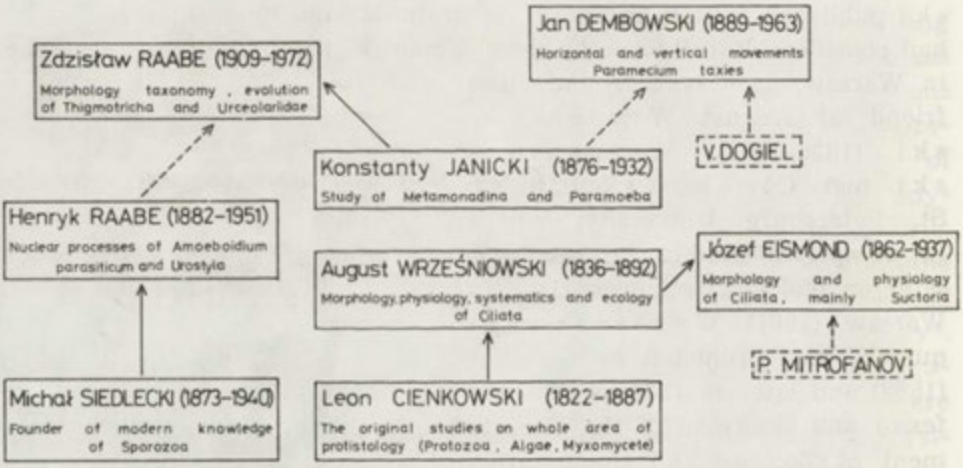

Fig. 3. The main founders of the protozoology in Poland

One of Wrześniowski's students was Józef Eismond (1862–1937), author of papers on morphology and physiology of ciliates, mainly *Suctoria*. After Wrześniowski retired in 1889, Eismond moved from the Department of Zoology to the Department of Comparative Anatomy and Embryology at the Tsar Warsaw University where the chairman (1890–1915) was P. Mitrofanov, a Russian well disposed to the Polish assistants and students. Under the Mitrofanov's chairmanship protozoological studies were carried out by Józef Eismond, Jan Sosnowski, Mieczysław Konopacki, Mieczysław Kowalewski and others.

At the end of the XIX century and at the beginning on the XX century the most successful protozoological studies were performed by Poles: Michał Siedlecki, Konstanty Janicki, Teodor

Viwieger, Juliusz Zweibaum and Romuald Minkiewicz at the foreign universities and scientific institutions. Michał Siedlecki (1873–1940) after Ph. D.'s promotion at the Jagiellonian University in Cracow spent three years (1896–1899) abroad at the Berlin University, the Naples Zoological Laboratory and Pasteur Institute and than moved back to Jagiellonian University. The years between 1896–1904 were exceedingly productive. At the Department of Zoology of Berlin University Siedlecki and Fritz Schaudinn discovered (1897) fertilization phenomena in *Coccidia*. During the next seven years Siedlecki as a result of his own cytobiological investigation, described the life cycles of several species of *Coccidia*, especially *Aggregata eberthi* (*Closia actopiana*) and Gregarine (*Monocystis ascidiae*).

Fig. 4. Michał Siedlecki (1873–1940)

Professor Siedlecki, one of the most famous Polish biologists in the first half of the XX century, published the last protozoological paper in 1911 and than shifted his own research to the ichthiology, oceanobiology and the problems of nature protection. He influenced indirectly the further development of protozoology by one of his students, Henryk Raabe (1882–1951), the father of Zdzisław Raabe (1909–1972). Henryk Raabe reported new data on nuclear processes of *Amoebidium parasiticum* and *Urostyla grandis*.

Konstanty Janicki (1876–1932) was a world wide known investigator of protozoa belonging to *Metamonadina* and genus *Paramoeba* and life cycles of tapeworms *Diphilobothrium latum* and *Amphilina*. Janicki worked at Basilea University and in 1919 was nominated a professor and chairman of the Department of Zoology in restituted Polish Warsaw University.

Protozoology got a new stimulus after Poland regained independence in 1918. Like Konstanty Janicki, also returned from abroad to Poland Teodor Viwieger, Romuald Minkiewicz, Józef Eismond, Juliusz Zweibaum, Jan Dembowski, Wiktoria Stanisława Świnarska (Dembowska) and they offered their knowledge, experience and enthusiasm.

All of them worked for some time at the M. Nencki Institute of Experimental Biology, which was organized in 1918–1919. Nencki Institute became shortly, a main research center in the field of protozoology as a result of Dembowski's and his students activity.

Fig. 5. Konstanty Janicki (1876-1932)

Jan Dembowski (1889–1963) began his scientific research in the Institute of Invertebrates Zoology at the University of St. Petersburg, guided by W. A. Dogiel. Later on he specialized in Vienna in the Laboratory of H. Przibram. After he came to Warsaw he married Wiktoria Stanisława Świnarska, who became his nearest friend and co-worker. Dembowska (1891–1962) performed at the Nencki Institute the fundamental research on regeneration of *Stylonychia mytilus* and in marine *Hypotricha*.

Dembowski was graduated as a Ph. D. (promoter K. Janicki) at the University of Warsaw in 1920.

About this time two main lines of his scientific interest have been established for all his life: the physiology of *Paramecium* and ethology of insect, crabs and mammales.

Dembowski studied experimentally food preference, motor response to stimuli and geotaxis of *Paramecium*.

He showed that the theory of the statocyst is unconvincing and found that the center of gravity of *P. caudatum's* body is shifted posteriorly and that the posterior, heavier half of the ciliate's body constitutes a subtle means of its orintation.

Researches on *Paramecium* of Dembowski students, especially Max Cheifec's and Wanda Milicer's were very impressive. In 1934, Dembowski was appointed a professor at the Department of Biology, Stefan Batory University in Wilno. In Wilno, he organized a very well equipped laboratory where he employed numerous young students. Most of the achievements of the Polish science were destroyed during the second World War.

Dembowski and his wife survived occupation and returned to the Nencki Institute, temporarily reestablished in Łódź. The most comprehensive development of protozoology in Poland took place during the last three decades. Jan Dembowski, Zdzisław Raabe and theirs students had the main share in it. Specially effective was

the contribution of Dembowski to the reconstruction of the Nencki Institute education of followers in field of protozoology like: M. Brutkowska, M. Doroszewski, S. Dryl, A. Grębecki, L. Kuźnicki, W. Kinastowski and organization of the polish science. He prepared and presided over the First Congress of the Polish Science (1951). On the basis of this Congress resolution, the Polish Academy of

Fig. 6. Jan Dembowski, Wiktoria Stanisława Dembowska and Leszek Kuźnicki in the Department of Experimental Biology, University of Łódź, September 1952

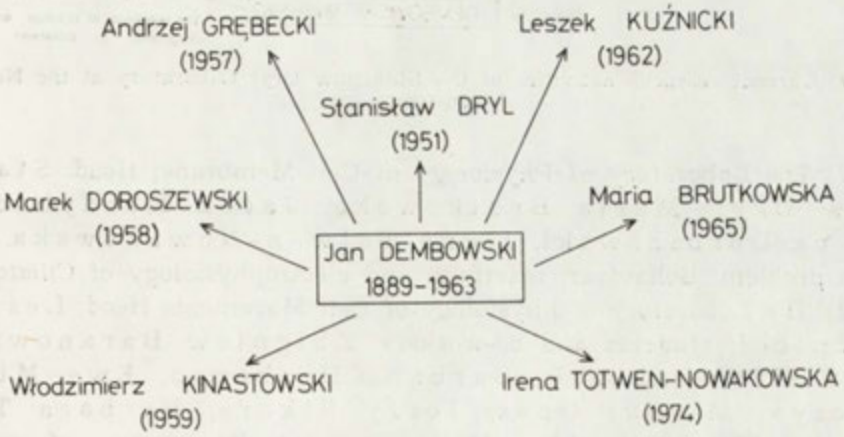

Fig. 7. Jan Dembowski post-war students on the field of protozoology. In parenthesis the year of receiving Ph. D's

Sciences was founded and Dembowski was elected its first President (1952–1957).

The Polish Academy of Science, became in time the major institution which sponsored the basic researches, catered also the development of protozoology, especially that the Nencki Institute became one of its scientific institutions.

Dembowski during his whole life published nine books among others, a classical work in Polish literature on *Paramecium* entitled, "The Natural History of Protozoan" (first ed. in 1924, fifth in 1962).

The Latest Years

After Dembowski retired in 1961, one of his post-war students S. Dryl, was his successor as the Head of the Department of Biology. During the last twenty years the Department changed the name to the Department of Cell Biology and it is divided now into five laboratories.

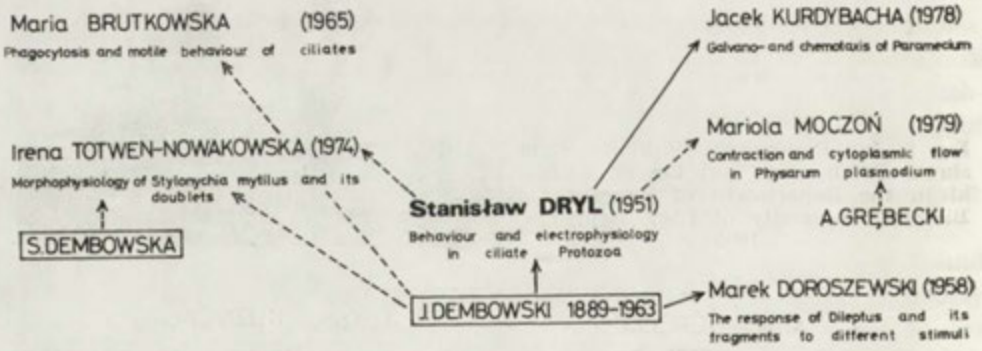

Fig. 8. Current research activities of the Stanisław Dryl Laboratory at the Nencki Institute

(1) The Laboratory of Physiology of Cell Membrane; Head: Stanisław Dryl, Maria Brutkowska, Jacek Kurdybacha, Marek Doroszewski, Irena Totwen-Nowakowska. The main problem: Behaviour, reactivity and electrophysiology of *Ciliata*.

(2) The Laboratory of Physiology of Cell Movements Head: Leszek Kuźnicki; students and co-workers: Zbigniew Baranowski, Stanisław Fabczak, Barbara Hrebenda, Ewa Mikołajczyk, Michał Opas, Jerzy Sikora, Barbara Tołłoczko. The main problem: Mechanisms and ultrastructure of various types of primitive motile phenomena.

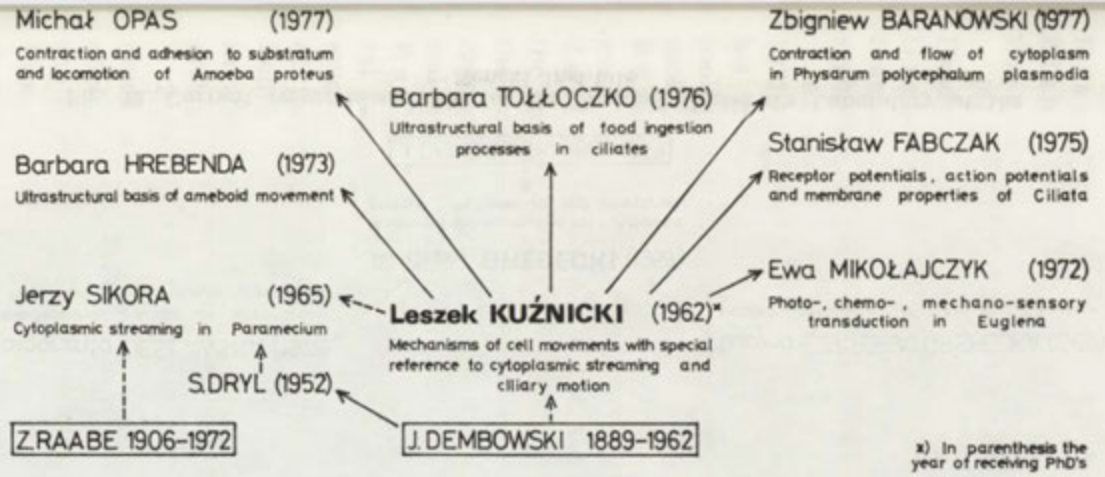

Fig. 9. Current research activity of the Leszek Kuźnicki's Laboratory at the Nencki Institute

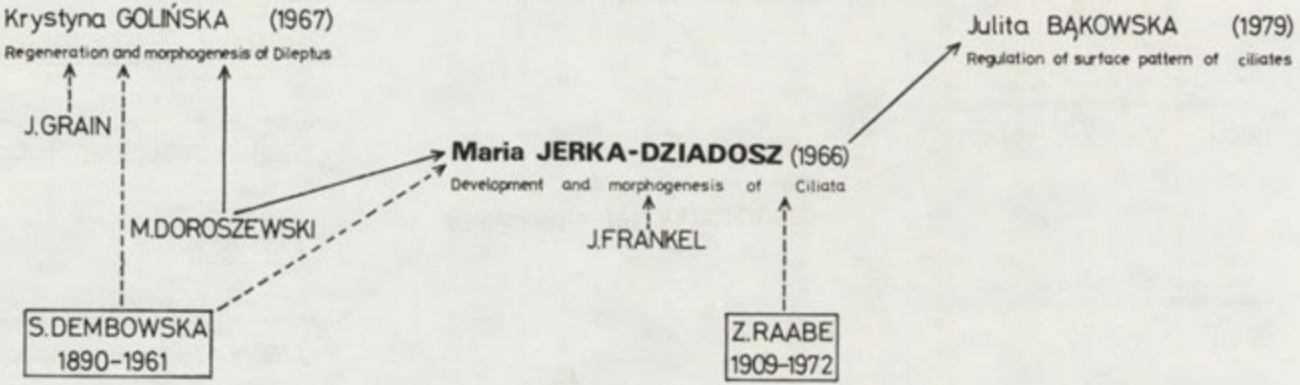

Fig. 10. Current research activities of the Laboratory of Regeneration and Morphogenesis of Protozoa. Head: Maria Jerka-Dziadosz

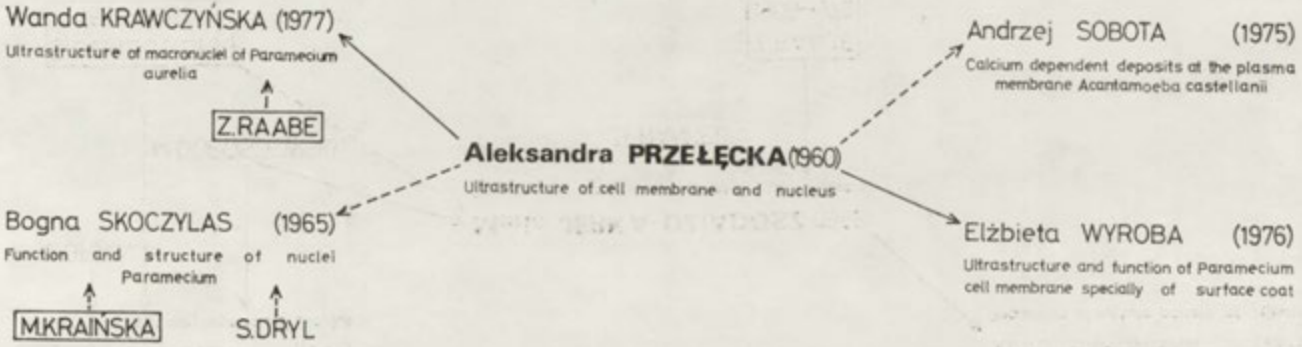

Fig. 11. Current research activities in field of protozoology of Laboratory of Cytochemistry at Nencki Institute. Head: Aleksandra Przełęcka

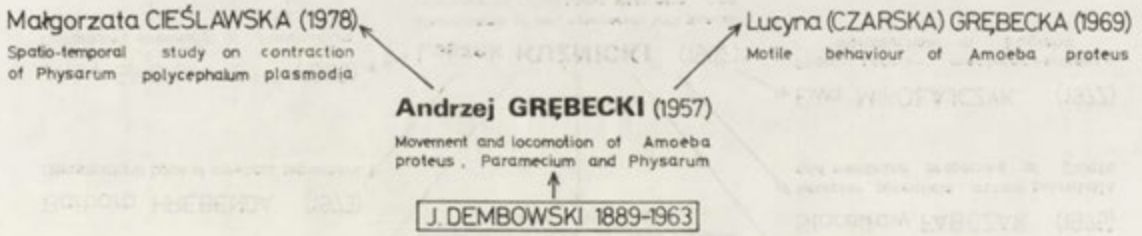

Fig. 12. Current research activities of the Andrzej Grębecki's Laboratory at the Nencki Institute

(3) The Laboratory of Regeneration and Morphogenesis of *Protozoa*; Head: Maria Jerka-Dziadosz, staff: Krystyna Golińska, Julita Bąkowska; The main problem: Development of ciliates.

(4) The Laboratory of Cytochemistry of Growth and Differentiation Processes. Head: Aleksandra Przełęcka, staff: Wanda Krawczyńska, Bogna Skoczylas, Andrzej Sobota, Elżbieta Wyroba; The main problem: Ultrastructure and function of cell membrane and nucleus.

(5) The Laboratory of Morphodynamic of Simple Motile Systems. Head: Andrzej Grębecki, students and co-workers: Lucyna Grębecka, Małgorzata Cieślawska; The main problem: Mechanism of amoeboid motion of *Amoeba proteus* and *Physarum polycephalum*.

After Dembowski retired (1961) Zdzisław Raabe (1909–1972) was the leader of the polish protozoologists. He was inspired by his father Henryk Raabe to study zoology and especially protozoology. He studied at the Warsaw University, where he got the protozoological background from Konstanty Janicki. Zdzisław Raabe studied parasitic ciliates during more than forty years, however, the most productive were the latest two decades of his life. In 1953 Zdzisław Raabe started to work at the Warsaw University as the professor and chairman of the Department of Zoology and stayed on the position until he suddenly died in 1972. He described a number of species and contributed to the knowledge of morphology, morphogenesis and systematics mainly of *Thigmotricha* and *Urceolariidae*. Zdzisław Raabe gave also attention to general taxonomy and phylogenetic trends among *Protozoa*. He was the author of the first textbook for students in Polish "Outline of Protozoology" (1964) in which he presented his idea on the system of *Protozoa*.

Zdzisław Raabe contributed much to the international collaboration among protozoologists. He was one of the initiators of the First International Protozoological Congress in Prague in August 1961, although progressive illness prevented him from coming to Prague. In Prague as well as in all successive International Protozoological Congresses numerous g-oup of Poles actively participated. As a member of

Fig. 13. Zdzisław Raabe (1909–1972)

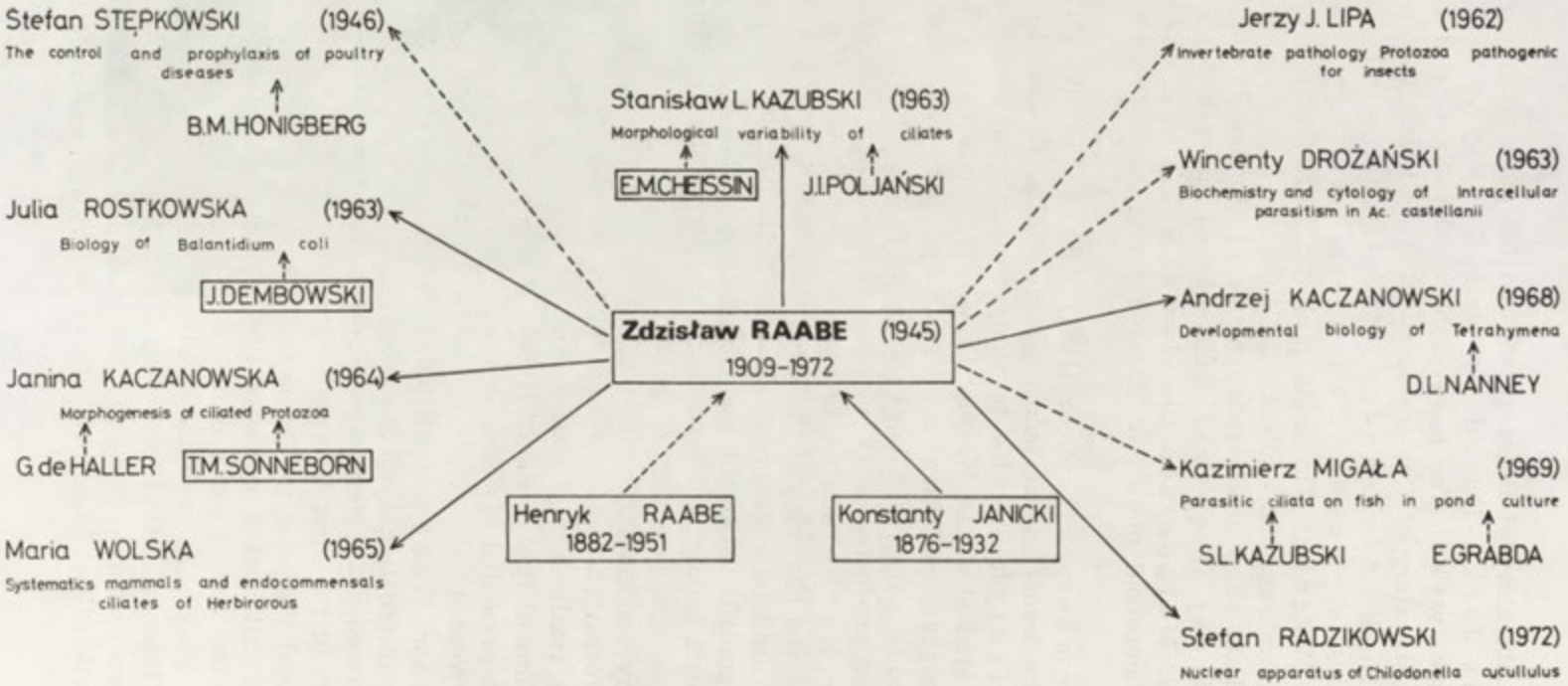

Fig. 14. Current research activities Zdzisław Raabe students

the International Commision of Protozoology he took part in the organization and participation of the Second Protozoological Congress in London of the third one in Leningrad.

The new international journal "Acta Protozoologica" edited by the Nencki Institute established in 1962 was one of the positive aspects of the international cooperation, initiated by Zdzisław Raabe. He was an editor in chief of nine volumenes (1963–1972) of "Acta Protozoologica".

Zdzisław Raabe was an excellent teacher. He directed the researches of many students, more than twenty completed Ph. D.'s under his guidance. Some of them carried out at present protozoological studies at various universities and scientific institutions. At the Institute of Zoology at the Warsaw University Janina Kaczanowska, Andrzej Kaczanowski, Stefan Radzikowski carry out morphogenetic work with ciliates, Stanisław Kazubski is studying morphological variability among some species of parasitic *Ciliata* at the Institute of Parasitology of the PAS.

Among Zdzisław Raabe students, the current study on parasitic and commensalic protozoa are carried out by: Jerzy J. Lipa — the head of Laboratory of Insect Pathology at the Institute of Plant Protection in Poznań, Kazimierz Migała; the head of Fishery Laboratory, at the Research Institute on Environmental Development, Julia Rostkowska[1] the head of Department of General Zoology and Parasitology at the Silesian University in Katowice and Maria Wolska from University of Łódź.

Besides the above mentioned problems, recently the following problems have been taken up in the field of basic protozoology.

— Systematic genetics and karyology of *Paramecium* — Zofia Komala, Halina Kościuszko and Ewa Przyboś (Department of Systematic and Experimental Zoology, PAS, Cracow).
— Systematics and ecology of free living ciliates — Anna Czapik (Jagiellonian University), Elżbieta Grabacka (Lab. of Water Biology PAS, Cracow).
— Systematics of *Euglenoidina parasitica* — Włodzimierz Michajłow, Irena Wita (Institute of Parasitology PAS).
— *Mitochondria* of *Acanthamoeba* — Jan Michejda, L. Hryniewiecka (A. Mickiewicz University of Biology, Poznań).
— Biochemistry and cytology of intracellular parasitism — Wincenty Drożański (Maria Curie-Skłodowska University, Lublin).
— Host-parasite relationship — Tadeusz Dzbeński (State Institute of Hygiene, Warsaw).

[1] Dr Julia Rostkowska died on 10th June 1982.

— Biology of parasitic amoeba — Witold Kasprzak (Academy of Medicine, Poznań).
— Ecology and bioenergetics of *Protozoa* — Romuald Klekowski (Institute of Ecology of PAS, Dziekanów/Warsaw).
— A role of the plasma membrane in regulation of cellular movement and metabolism — Włodzimierz Korohoda (Jagiellonian University).
— Mutualistic relationship between protozoa and fungi; toxoplasmosis — Alicja Kurnatowska (Medical Academy, Łódź).
— *Amoebiasis*, serology of parasitic diseases — Przemysław Myjak (Institute of Marine and Tropical Medicine, Gdynia).
— Photobiology and trophic relations between two partners of symbiosis — Ryszard Pado (The Higher Pedagogical School, Cracow).
— Upper cretaceous and tertiary *Foraminifera* — Krystyna Pożaryska (Institute of Paleobiology of PAS).
— *Coccidiasis* — Janina Pastuszko (Agricultural Academy of Warsaw).
— Prophylaxis of poultry diseases — Stefan Stępkowski (Agricultural Academy in Lublin).

The time limit of the lecture forces me to shorten the amount of information and for this reason, I could not present all the trends, conceptual aspects and individual activities of past and present Polish protozoologists. I would recomend anyone interested in this area to the enclosed Authors Catalogue where the most, in my opinion, meaningful papers, are listed. This bibliography covers only about half papers written by Poles in widely known languages, but gave a general picture of protozoological studies in our country in the period from 1855 to January 1, 1980.

Looking back at the state in 1951, or even in 1961 one could say, that in last twenty, thirty years protozoology in Poland has evident scientific, editorial and organizational successes. In my opinion these achievements are still below our whishing, less our ability. In the enclosed Author Catalogue of the most active protozoologists with Ph. D.'s degree you'll find the majority of young scientists. Outside this list remains a considerable number of talented young people who just start their scientific work.

I hope that the new generation has a real chance to make our dream of fully advanced protozoology in Poland true.

AUTHOR CATALOGUE

Part I — Past

Chejfec Maks

1929 — Długość życia *Paramecium caudatum* w związku z odżywianiem. Acta Biol. Exp., 4, 73–118.

1930 — Zur Kenntnis der Kernreorganisationprozesse bei *Paramecium caudatum*. Arch. Protistenk., 70, 87–118.

1933 — Die experimentellen Grenzwerte des Lebens von Protozoen auf Grund der Untersuchungen des *Paramecium caudatum*. Arch. Protistenk., 79, 467–478.

1935 — Das Verhalten von *Paramecium caudatum* in Glukoselösungen. Acta Biol. Exp., 9, 69–90.

Cienkowski Leon

1855 — Bemerkungen über Stein's Acineten-Lehre. Bull. phys. mat. de l'Acad. Imper. Sci. de St. Petersburg, 13, 19. Mélanges Biol. tir. Bull. phys.-mat. l'Acad. Imper. Sci. de St. Petersburg, 2, 3, 263–272.

1855 — Algologische Studien. Bot. Zeit., 4, 5, 777–782, 801–806.

1855 — Über Cystenbildung bei Infusorien. Z. wiss. Zool., 4, 301–306.

1858 — Die Pseudogonidien. Jahrb. wissen. Bot., 1, 371–376.

1861 — Ueber parasitische Schläuche auf Crustaceen und einigen Insectenlarven (*Amoebidium parasiticum*). Bot. Zeit., 19, 25, 169–174.

1862 — Zur Entwicklungsgeschichte der Myxomycete. Jahrb. wissen. Bot., 3, 325–337.

1863 — Das Plasmodia. Jahrb. wissen. Bot., 3, 440–441.

1865 — Ueber einige chlorophyllhalting Gloeocapsen. Bot. Zeit., 3, 21–27.

1865 — Beiträge zur Kenntnis der Monaden. Arch. Microsk. Anat., 1, 202–232.

1867 — Ueber den Bau und die Entwicklung der Labyrinthuleen. Arch. Microsk. Anat., 3, 274–310.

1867 — Ueber die *Clathrulina*, eine neue Actinophryen-Gattung. Arch. Microsk. Anat., 3, 311–316.

1870 — Ueber Palmellaceen und einige Flagellaten. Arch. Microsk. Anat., 4, 421–438.

1871 — Ueber Schwärmerbildung bei *Noctiluca miliaris*. Arch. Microsk. Anat., 7, 131–139.

1871 — Ueber Schwärmerbildung bei Radiolarien. Arch. Microsk. Anat., 7, 372–381.

1873 — Ueber *Noctiluca miliaris*. Arch. Microsk. Anat., 9, 47–63.

1876 — Ueber einige Rhizopoden und verwandte Organismen. Arch. Microsk. Anat., 12, 15–50.

Dembowska Wiktoria, Stanisława

1925 — Studien über die Regeneration von *Stylonychia mytilus*. Arch. Mikrosk. Anat. EntwMech., 104, 185–209.

1926 — Studies on the regeneration of *Protozoa*. II. Regeneration of ciliary apparatus in some marine *Hypotricha*. J. Exp. Zool., 43, 485–504.

1938 — Korperreorganisation von *Stylonychia mytilus* bei Hungern. Arch. Protistenk., 91, 89–105.

Dembowski Jan

1913 — Versuche über die Merotomie der Gregarinen. Arch. Protistenk., 29, 1–21.

1924 — Über die Bewegungen von *Paramecium caudatum*. Arch. Protistenk., 47, 25–54.

1929 — Die Vertikalbewegungen von *Paramecium caudatum*. I. Die Lage des Gleichgewichtszentrums im Körper des Infusorien. Arch. Protistenk., 66, 104–132.

1929 — Die Vertikalbewegungen von *Paramecium caudatum*. II. Einfluss einiger Aussenfaktoren. Arch. Protistenk., 68, 215–261.

1931 — Die Vertikalbewegungen von *Paramecium caudatum*. III. Polemisches und Experimentalles. Arch. Protistenk., 74, 153–187.

1938 — Über die Rhytmik der Parameciumteilungen. Acta Biol. Exp., 12, 22–33.

1924 — Adaptacija infuzorij k solam odnovalentnych metałlov. Izvest. Akad. Nauk SSSR Otdiel. biol. nauk., 3, 181–185.

1950 — On conditioned reactions of *Paramecium caudatum* towards light. Acta Biol. Exp., 15, 5–17.

1950 — Lubocka A. and Dembowski J. — Circulation of food vacuole in *Paramecium caudatum*. Acta Biol. Exp., 15, 19–35.

Drzewiecki Wojciech

1903 — Ueber vegetative Vorgänge im Kern und Plasma der Gregarinen des Regenwurmkodens. Arch. Protistenk., 3, 207–225.

1907 — Ueber vegetative Vorgänge im Kern und Plasma der Gregarinen. 2. *Stomatophora coronata* nov. gen. (*Monocystis coronata* Hesse). Arch. Protistenk., 10, 216–246.

Eisenberg-Hamburg Eliza

1926 — Recherches sur le fonctionnement de la vesicule des infusoires dans les conditions normales et sous l'actions de certains agents experimentaux: pression osmotique et electrolites. Arch. biol., 35, 441–464.

1929 — Recherches comparatives sur le fonctionnement de la vacuole pulsatile chez les infusoires parasites de la grenouille et chez les infusoires d'eau douce: Influence de la pression osmotique, des électrolytes et du pH. Arch. Protistenk., 68, 451–470.

1932 — Einfluss der Sr-Salze auf die Bewegung von *Paramecium caudatum*. Die Rolle des Ca und der Konzentration der Wasserstoffionen. Arch. Protistenk., 77, 108–124.

Eismond Józef

1890 — Eine einfache Untersuchungsmethode für Vebende Infusorien. Zool. Anz. (Leipzig) 13, 723–724.

1896 — O dopolnenie k'voprosu o delenii kletočnovo tela. Trudy Obščestva Estestvoispytatelej pri Imperatorskom Varšavskom Universitete. t. III, 1–20.

1899 — Ueber Zellteilung. In: Comptes rendus des XII Congres Inter. de Medicine, II, Sec. I, Moscow, 112–115.

Hoyer Henryk F.

1864 — Trawienie oscillatoriów w amoebach. Pam. Tow. Lek. Wet. Warszawa, 1864. T. LI, Nr 2, str. 412.

1889 — Ueber das Verhalten der Kerne bei der Conjugation des Infusores *Colpidium Colpoda*. St. Vorgetragen in der Sitzung der mathemnaturwissen Klasse der polnische Akademie der Wissenschaften in Krakau am 6 Februar 1889. Arch. Mikrosk. Anat. EntwMech., Gesch., 93–134.

Janicki Konstanty

1908 — Contribuzione alla conoscenza di alcuni protozoi parassiti della *Periplaneta orientalis* (*Lophomonas blattarum*, Stein; *L. striata* Bütschli; *Amoeba blattae* Bütschli). Atti R. Accad. Lincei, Roma. Rendie. Cl. Sc. Fis., Mat. e Nat., an. 305, 5, 17, 140–151.

1909 — Ueber Kern und Kernteilung bei *Entamoeba blattae* Bütschli. Biol. Zbl., 29, 381–393.

1910 — Untersuchungen an parasitischen Flagellaten. 1 Teil. *Lophomonas blattarum* Stein. *L. striata* Bütschli. Z. Wiss. Zool., 95, 243–315.

1911 — Zur Kenntnis des Parabasalapparats bei parasitische Flagellaten. Biol. Zbl., 31, 11, 321–330.

1912 — Bemerkungen zum Kernteilungsvorgang bei Flagellaten, namentlich bei parasitischen Formen. Verh. schweiz. naturf. Ges., 23, 82–111.

1912 — Paramoebenstudien. (*P. pigmentifera* Grassi und *P. chaetognathi* Grassi). Z. Wiss. Zool., 103, 449–518.

1912 — Untersuchungen an parasitischen Arten der Gattung *Paramoeba* Schaudinn. (*P. pigmentifera* Grassi und *P. chaetognathi* Grassi) Verh. schweiz. naturf. Ges., 23, 6–21.

1915 — Untersuchungen an parasitischen Flagellaten. 2 Teil. Die Gattung *Devescovina, Parajoenia, Stephanonympha, Calonympha*. Ueber den Parabasalapparat. Ueber Kernkonstitution und Kernteilung. Z. Wiss. Zool., 112, 573–691.

1928 — Studien am Genus *Paramoeba* Schaud. Neue Folge, I. Teil. Z. Wiss. Zool., 131, 588–644.

1932 — Studien am Genus *Paramoeba* Schaud. Neue Folge, II. Teil. Ueber das *Trichopodium*, nebst einer Ergänzung zum. I. Teil. Z. Wiss. Zool., Abt. A., 142, 587–623.

Kinastowski Włodzimierz

1963 — Der Einfluss der mechanischen Reize auf die Kontraktilität von *Spirostomum ambiguum* Ehrbg. Acta Protozool., 1, 201–222.

1963 — Das Problem "des Lernens" bei *Spirostomum ambiguum* Ehrbg. Acta Protozool., 1, 223–236.

Lewkowicz Ksawery

1897 — Ueber den Entwicklungsgang und die Einteilung der Malariaparasiten. Zentbl. Bakt., 1, v. 21, 6 Febr. pp. 129–133.

1898 — Ueber Phenocoll. Analhen, Chinopyrin und Euchinin als antimalarische Mittel. Wien. Klin. Wochnschr., v. 11–41, 13 Oct. pp. 922–924.

1898 — *Echinococcus* der Niese. Klin. Wochnschr., 5, 10, 513–514.

1900 — Zur Biologie der Malariaparasiten. Wien. Klin. Wochnschr., 13 (9) 206–209, 233–246.

Milicer Wanda

1935 — Recherches experimentales sur le systeme neuro-moteur de *Paramecium caudatum*. Acta Biol. Exp., 9, 174–193.

Minkiewicz Romuald

1901 — Issledovanija nad prostieišimi Černogo moria. 1. Organizatsija, razmnoženie i položenie v sistemie roda *Euplotes* Ehrbg. (Russian text). Trudy Obsh. Estestv. Imp. Kazansk. Univ., 35 (1), 1–67.

1902 — Bemerkungen zur Arbeit von Atsuschi Yasuda: Studien über die Anpassungsfähigkeit einiger Infusorien an concentrierte Lösungen. Zool. Anz., Leipzig, 25, 3, 124–125.

1912 — Un cas de reproduction extraordinaire chez un protiste, *Polyspira delagei* Minkiew. Compt. Rend. Soc. Biol., Paris, 155 (16), 733–737.

1912 — Ciliata chromatophora, nouvel ordre d'infusoires à morphologie et reproduction bizarres. Compt. Rend. Soc. Biol., Paris, 155 (10), 513–515.

1913 — Studya nad wymoczkami o konjugacyi łańcuchowej. Studes sur les infusoires syndesmogames, à gamontes et gamètes (French text). Bull. Int. Acad. Pol. Sci. Lett., Cracovie, Cl. Sc. Math. et Nat., Ser. B, Sci. Nat., (8), 742–749.

Pigoń Andrzej

1959 — Pigoń A. and Edström — Nucleic acid changes during starvation and encystment in a ciliate (*Urostyla*). Exp. Cell Res., 18, 648–656.

1960 — Changes of enzyme activity during starvation and encystment of a ciliate *Urostyla*. Amylase, Catalase. Acta Biol. Cracov., Ser. Zool., 3, 59–70.

1961 — Changes in the respiratory activity during starvation and encystment of a ciliate *Urostyla*. Acta Biol. Cracov., Ser. Zool., 4, 33–45.

1961 — Changes of enzyme activity during starvation and encystment of a ciliate *Urostyla*. Dipeptidase, Amino acid oxidase. Acta Biol. Cracov., Ser. Zool., 4, 123–142.

1962 — Changes of enzyme activity during starvation and encystment of a ciliate (*Urostyla*). Cathepsin, Acid Phosphate. Acta Biol. Cracov., 5, 175–197.

Przesmycki Marian

1894 — Über die Zellkörnchen bei den Protozoen. Biol. Zbl., 14, 620–626.

1897 — Ueber die intravitale Färbung des Kerns und des Protoplasmas. Biol. Zbl., 12, 9, 321–33, 10, 353–364.

1901 — Ueber parasitische Protozoen aus dem Inneren der Rotatorien, Bull. Int. Acad. Pol. Sci. Lett., Cracovie Cl. Sc. Math. Nat., (7), 358–408.

Raabe Janina

1959 — Raabe J. and Raabe Z. — *Urceolariidae* of molluscs of the Baltic Sea. Acta Parasit. Pol., 7, 453–465.

1961 — Raabe J. and Raabe Z. — *Urceolariidae* from fresh-water and terrestrial molluscs in Poland. Acta Parasit. Pol., 9, 141–152.

Raabe Henryk

1912 — Les divisions du noyau chez *Amoebidium parasiticum* Cienk. Arch. Zool. exp. gén., 50, 371–398, 19, 1–45.

1916 — Les générations automnales chez l'*Amoebidium parasiticum* Cienk. Traveux Soc. Sci. de Varsovie.

1923 — La signification de la concentration en ions H, de la quantité de nourriture et du rapport de la surface de la culture a son volume,

dans le developpement du flagelle *Prowazekia* (Bodo) *edax*. Compt. Rend. Soc. Biol., Paris, 89, 1351–1353.

1925 — *Amoeba unduligrada* sp. nov. Structure de l'amibe et division du noyau. Arch. Zool. exp. gén., 64, 3, 327–351.

1925 — Sur l'influence de certaines facteurs dans le développement du flagellé *Prowazekia* (Bodo) *edax*. I partie. Bull. Soc. Chim. Biol., 7, Paris.

1925 — Sur l'influence de certains facteurs dans développement du flagellé *Prowazekia* (Bodo) *edax*. II partie. Bull. Soc. Chim. Biol., 7, Paris.

1946 — Aparat jądrowy *Urostyla grandis* Ehrbg. Część I. Aparat jądra małego. L'appareil nucléaire d'*Urostyla grandis* Ehrbg. Part. I. L'appareil micronucléaire. Annls. Univ. M. Curie-Skłodowska, Sect. C, I, 6, Lublin, 1–34.

1947 — Aparat jądrowy *Urostyla grandis* Ehrbg. Część II. Aparat jądra dużego. L'appareil d'*Urostyla grandis* Ehrbg. Part. II. L'appareil macronucléaire. Annls. Univ. M. Curie-Skłodowska, Sect. C, I, 6, Lublin, 133–170.

1950 — *Amoeba vespertilio* Penard; structure, fission of the amoeba and nucleus; biology. Bull. Int. Acad. Pol. Sci. Lett. Cracovie Cl. Sci. Math. Nat. Ser. B, II, 353–375.

Raabe Zdzisław

1933 — Untersuchungen an einigen Arten des Genus *Conchophthirus* Stein. Bull. Acad. Pol. Sci. Lett., Cracovie, Sér. B. 1932, 295–310.

1934 — Weitere Untersuchungen an einigen Arten des Genus *Conchophthirus* Stein. Mem. Acad. Pol. Sci. Lett., Sér. B, 221–235.

1935 — Weitere Untersuchungen an parasitische Ciliaten aus dem polnischen Teil der Ostsee. I. Cilia *Thigmotricha* aus den Familien: *Thigmophryidae, Conchophthiridae* und *Ancistrumidae*. Annls. Mus. zool., pol., 11, 419–442.

1938 — Weitere Untersuchungen an parasitischen Ciliaten aus dem polnischen Teil der Ostsee. II. Ciliata *Thigmotricha* aus den Familien: *Hypocomidae* Bütschli und *Sphaenophryidae* Ch. et Lw. Annls. Mus. zool., pol., 13, 41–75.

1947 — Recherches sur les ciliés Thigmotriches (*Thigmotricha* Ch. Lw.). I. Sur un genre nouveau de la famille *Conchophthiridae* Kahl. Annls. Univ. M. Curie-Skłodowska, Sect. C, 1, 61–70.

1947 — Recherches sur les ciliés Thigmotriches (*Thigmotricha* Ch. Lw.). II. Espèce nouvelle d'eau douce du genre *Ancistrina* Cheissin. Annls. Univ. M. Curie-Skłodowska, Sect. C, 2, 111–120.

1949 — Recherches sur les ciliés Thigmotriches (*Thigmotricha* Ch. Lw.) III. Développment non-parallèle de deux espèces du genre *Sphenophrya* Ch. Lw. Annls. Univ. M. Curie-Skłodowska, Sect. C. 4, 119–135.

1949 — Recherches sur les ciliés Thigmotriches (*Thigmotricha* Ch. Lw.). IV. Position systematique du genre *Protophrya* Kofoid. Annls. Univ. M. Curie-Skłodowska, Sect. C, 4, 195–201.

1950 — Recherches sur les ciliés Thigmotriches (*Thigmotricha* Ch. Lw.). V. Ciliés Thigmotriches du lac Balaton (Hongrie). Annls. Univ. M. Curie-Skłodowska, Sect. C, 5, 197–215.

1958 — On some species of *Trichodina* (*Ciliata-Peritricha*) of gills of Adriatic fishes. Acta Parasit. pol., 6, 355–362.

1959 — Recherches sur les ciliés Thigmotriches. VI. Sur les genres "*Ancistruma*", "*Ancistrima*" et les genres voisins. Acta Parasit. pol., 7, 215–247.

1959 — *See*: Raabe J. and Raabe Z.
1961 — *See*: Raabe J. and Raabe Z.
1963 — Systematics of the family *Urceolariidae* Dujardin 1841. Acta Protozool., 1, 121–138.
1964 — Remarks on the principles and outline of the system of *Protozoa*. Acta Protozool., 2, 1–8.
1964 — The taxonomic position and rank of *Peritricha*. Acta Protozool., 2, 19–32.
1967 — Ordo *Thigmotricha* (*Ciliata–Holotricha*). I. Acta Protozool., 5, 1–36.
1970 — Ordo *Thigmotricha* (*Ciliata-Holotricha*). II. Familiae *Hemispeiridae*. Acta Protozool., 7, 117–180.
1970 — Ordo *Thigmotricha* (*Ciliata–Holotricha*). III. Familiae *Ancistrocomidae* et *Sphenophryidae*. Acta Protozool., 7, 385–463.
1971 — The morphogenetic principles of Sewertzoff, their extension and application to *Protozoa*. Acta Protozool., 9, 1–22.
1971 — Ordo *Thigmotricha* (*Ciliata-Holotricha*). IV. Familiae *Thigmophryidae*. Acta Protozool., 9, 121–170.
1972 — Ordo *Thigmotricha* (*Ciliata-Holotricha*). V. Familiae *Hysterocinetidae* et *Protoanoplophryidae*. Acta Protozool., 10, 115–184.

Siedlecki Michał

1897 — Schaudinn F., Siedlecki M. — Beiträge zur Kenntniss der Coccidien. Verh. dtsch. Zool. Ges. Leipzig, 192–203.
1898 — Reproduction sexuée et cycle évolutif de la coccidie de la seiche (*Klossia octopiana* Schn.). Compt. Rend. Soc. Biol., (Paris), 10, 5, 540–543.
1898 — Reproduction sexuée et début de la sporulation chez la coccidie des tritons (*Coccidium proprium* Schn.). Compt. Rend. Soc. Biol., (Paris), 10, 5, 663–665.
1898 — Etude cytologique et cycle évolutif de la coccidie de la seiche. Ann. Inst. Pasteur, (Paris), 12, 799–836.
1899 — Etude cytologique et cycle évolutif de *Adelea ovata* Schneider. Ann. Inst. Pasteur, 13, 169–192.
1901 — Sur les rapports des grégarines avec l'épithélium intestinal. Compt. Rend. Soc. Biol., (Paris), 53, 81–83.
1901 — Contribution à l'étude des changements cellulaires provoqués par les grégarines. Arch. Anat. Micr., 4, 87–100.
1903 — Quelques observations dur le rôle des amibocytes dans le coelome d'un annélide. Ann. Inst. Pasteur, (Paris), 17, 449–462.

Sokólska Julia

1927 — Sur les composants lipoidifères du plasma du protozaire parasite *Opalina ranarum*. Purk et Val. Compt. Rend. Soc. Biol., (Paris), V. 96 (8), 570–572.

Sosnowski Jan

1899 — Untersuchungen über die Veränderungen des Geotropismus bei *Paramecium aurelia*. Bull. Int. Acad. Sci. Cracovie, 130–136.

Vieweger Teodor

1912 — Recherches sur la sensibilite des infusoires (alkaliooxytaxisme), les reflexes locomoteurs, l'action des sels. Arch. Biol. Liége, 27, 723–799.

Wilczyński Jan

1939 — Zur weiteren Analyse der "Lag-Periode" in der Parameciumteilungsrhythmik. Arch. Protistenk., 93, 87–104.

Wrzesniowski August

1862 — Observations sur quelques infusoires. Annls. Sci. Nat. Zool., 16, 8–9.
1867 — Przyczynek do historyi naturalnej wymoczków (Beiträg zur Naturgeschichte der Infusorien), Kraków 1867, C. K. Uniwer. Druk. 7, 1–116.
1869 — Ein Beitrag zur Anatomie der Infusorien. Arch. Mikrosk. Anat., 5, 25–48.
1870 — Beobachtungen über Infusorien aus der Umgebung von Warschau. Z. Wiss. Zool., 20, 4.
1877 — Beiträge zur Naturgeschichte der Infusorien. Z. Wiss. Zool., 29.

Zweibaum Juliusz

1912 — Les conditions néessaires et suffisantes pour la conjugaison du *Paramecium caudatum*. Arch. Protistenk., 26, 275–393.
1921 — Ricerche sperimentali sulla conjugazione degli Infusori. I. Influenza della conjugazione sull' assorbimento dell' O_2 nel *Paramecium caudatum*. Arch. Protistenk., 44, 99–114.
1922 — Ricerche sperimentalle sulla conjugazione degli Infusori. II. Influenza della conjugazione sulla produzione dei materiale di riversa nel *Paramecium caudatum*. Arch. Protistenk., 44, 375–396.

AUTHOR CATALOGUE

Part II — Present

Balcerzak Czesław

1978 — Balcerzak Cz. and Rostkowska J. — The influence of gamma radiation on sensitivity of *Spirostomum ambiguum* to various sodium chloride concentrations and the effect of radioprotective substances. Acta Protozool., 17, 509–514.
1979 — The radiation changes of activation energy of phosphate ions transport across the *Spirostomum ambiguum* membrane and accumulation of these ions in cells. Acta Protozool., 18, 333–340.

Brutkovska Maria

1967 — Immobilization effect of $NiCl_2$ and food vacuole formation in *Paramecium caudatum*. Bull. Acad. Pol. Sci., 15, 119–122.
1972 — Brutkowska M. and Dryl S. — Galvanotactic response and food vacuole formation in *Tetrahymena pyriformis*. Acta Protozool., 11, 402–405.
1976 — Brutkowska M. and Mehr K. — Effect of ionic detergents on phagocytic activity of *Tetrahymena pyriformis* and *Paramecium caudatum*. Acta Protozool., 15, 67–76.
1978 — *See*: Wyroba E. and Brutkowska M.

Czapik Anna

1965 — *Prorodon raabei* sp. n. et sa biologie. Acta Protozool., 3, 21–26.
1971 — Les observations sur *Platyophrya spumacola* Kahl. Acta Protozool., 8, 363–366.
1976 — *Strombidium grande* Levander. Acta Protozool., 15, 273–275.
1976 — Les observations sur les ciliés d'une mare. Acta Protozool., 15, 277–287.
1976 — Czapik A. and Jordan A. — Les ciliés psammophiles de la mer Baltique aux environs de Gdańsk. Part II. Acta Protozool., 15, 423–445.
1977 — Deux ciliés psammophiles nouveaux: *Hippocomos loricatus* gen. n. sp. n. et *Pleuronema tardum* sp. n. Acta. Protozool., 16, 157–163.

Doroszewski Marek

1963 — Some features of the ciliary activity in *Dileptus*. Acta Protozool., 1, 187–192.
1963 — The response of *Dileptus* and its fragments to the puncture. Acta Protozool., 1, 313–319.
1964 — *See*: Golińska K. and Doroszewski M.
1965 — The response of *Dileptus cygnus* to the bisection. Acta Protozool., 3, 175–182.
1967 — Doroszewski M. and Golińska K. — The behaviour of toxic trichocysts in the course of regeneration in *Dileptus cygnus* Clap et Lachm. Acta Protozool., 4, 343–351.
1968 — Responses to shake of water in the course of regeneration in *Dileptus cygnus*. Acta Protozool., 5, 291–296.
1970 — Responses of the ciliate *Dileptus* to mechanical stimuli. Acta Protozool., 7, 354–362.
1972 — The responses to bisections of dividing *Dileptus cygnus*. Acta Protozool., 10, 109–113.
1978 — Doroszewski M. and Dryl S. — Contribution to studies on localization of chemoreceptor properties of cell surface in *Dileptus cygnus*. Acta Protozool., 17, 561–565.

Drożański Wincenty

1955 — Fatal bacterial infection of soil amoeba., Acta Microbiol. Pol., 5, 315.
1963 — Studies on intracellular parasites of free-living amoeba. Acta Microbiol. Pol., 12, 3–8.
1963 — Observation on intracellular infection of amoebae by bacteria. Acta Microbiol. Pol., 12, 9–23.
1969 — Bacteriolytic enzyme produced by *Acanthamoeba castellanii*. Acta Microbiol. Pol., 18, 155–168.
1969 — Bacteriolytic spectrum of the enzyme produced by *Acanthamoeba castellanii*. Acta Microbiol. Pol., 18, 169–176.
1972 — The *in vitro* experiments on the fatal infection of *Amoebida* (Kent, 1880) by pathogenic bacterium. Acta Protozool., 10, 275–284.
1972 — Purification and properties of bacteriolytic enzyme produced by *Acanthamoeba castellanii*. Acta Microbiol. Pol., IV/91, 33–52.
1972 — Bacteriolytic enzyme from *Acanthamoeba castellanii*: Enzymic mode of action. Acta Microbiol. Pol., IV/91.
1978 — Activity and distribution of bacteriolytic N-acetylmuramidase during growth of *Acanthamoeba castellanii* in axenic culture. Acta Microbiol. Pol., 27, 243–256.

1978 — Two forms of bacteriolytic endo N-acetylmuramidase in *Acanthamoeba castellanii*. Acta Biochim. Pol., 25, 229–238.
1979 — Drożański W. and Chmielewski T. — Electron microscopic studies of *Acanthamoeba castellanii* infected with obligate intracellular bacterial parasite. Acta Microbiol. Pol., 28, 123–133.

Dryl Stanisław

1958 — Photographic registration of movement of protozoa. Bull. Acad. Pol. Sci., Ser. Biol., 6, 429–430.
1959 — Chemotactic and toxic effects of lower alcohol on *Paramecium caudatum*. Acta Biol. Exp., 19, 95–104.
1959 — Effects of adaptation to environment on chemotaxis of *Paramecium caudatum*. Acta Biol. Exp., 19, 83–93.
1961 — Chemotaxis in *Paramecium caudatum* as adaptive responses of organism to its environment. Acta Biol. Exp., 21, 75–83.
1963 — Oblique orientation of *Paramecium caudatum* in electric field. Acta Protozool., 1, 193–199.
1964 — [Kinosita H.], Dryl S. and Naitoh Y. — Spontaneous changes in membrane potential of *Paramecium caudatum* induced by barium and calcium ions. Bull. Acad. Pol. Sci., Cl. II, 12, 459–461.
1964 — [Kinosita H.], Dryl S. and Naitoh Y. — Changes in the membrane potential and the response to stimuli in *Paramecium*. J. Fac. Sci. Univ. Tokyo, Sec. IV, 10, 291–301.
1966 — Dryl S. and Grębecki A. — Progress in study of excitation and responses in ciliates. Protoplazma, 62, 255–284.
1972 — *See*: Brutkowska M. and Dryl S.
1975 — Behaviour and motor response of *Paramecium* — In: "*Paramecium* — Recent Survey" Ed. W. J. van Wagtendonk, Elsevier, Amsterdam.
1977 — Dryl S. and Totwen-Nowakowska I. — Contribution to studies on motor response of *Stylonychia mytilus* to mechanical and chemical stimuli. Acta Protozool., 16, 345–349.
1978 — *See*: Doroszewski M. and Dryl S.
1978 — Dryl S. and Kurdybacha J. — Dependence of chemotaxis in *Paramecium caudatum* and *Paramecium aurelia* 51 s on concentration of calcium ions in external medium. Acta Protozool., 17, 551–559.
1979 — Dryl and Hildebrand E. — Modifying effects of chemical factors on behavior and excitability of ciliate *Protozoa*. Acta Protozool., 18, 17–21.

Dzbeński Tadeusz H.

1974 — Exoantigens of *Trypanosoma cruzi in vivo*. Z. Tropenmed. Parasit., 25, 485–491.
1976 — Dzbeński T. H., Michalak T. and Płonka W. — Electron microscopic and radioisotopic studies on cap formation in *Toxoplasma gondii*. Infect. Immun., 14, 1196–1201.
1976 — Dzbeński T. H. and Zielińska E. — Antibody — induced formation of caps in *Toxoplasma gondii*. Experientia (Basel), 32, 454–455.

Fabczak Stanisław

1972 — *See*: Kuźnicki L., Sikora J. and Fabczak S.
1973 — Fabczak S., Walczak T. and Korohoda W. — Studies on

the electrical stimulation of contraction in *Spirostomum*. I. Conditions of the quantitative measurements. Cytobiologie, 7, 152–163.

1974 — Studies on the electrical stimulation of contraction in *Spirostomum*. II. The effect of external ions on cell excitability. Cytobiologie, 10, 131–139.

1977 — Studies on the electrical stimulation of contraction in *Spirostomum*. III. The effect of polyactions on cell membrane excitability. Acta Protozool., 16, 177–183.

Golińska Krystyna

1963 — Experimental study on rebounding from a mechanical obstacle in *Paramecium caudatum*. Acta Protozool., 1, 113–120.

1964 — Golińska K. and Doroszewski M. — The cell shape of *Dileptus* in the course of division and regeneration. Acta Protozool., 2, 59–67.

1965 — Macronucleus in *Dileptus cygnus* and its change in division. Acta Protozool., 3, 143–152.

1966 — Regeneration of anuclear fragments in *Dileptus cygnus* Clap. et Lachm. Acta Protozool., 4, 41–50.

1967 — *See*: Doroszewski M. and Golińska K.

1969 — Golińska K. and Grain J. — Observations sur les modifications ultrastructurales lors de la regeneration chez *Dileptus cygnus*. Clap. et Lachm. 1859. Cilié Holotriche Gymnostome. Protistologica, 5, 447–464.

1971 — Comparative studies on the morphology of *Dileptus anatinus* sp. n. (*Holotricha, Gymnostomata*). Acta Protozool., 8, 367–378.

1972 — Studies on stomatogenesis in *Dileptus* (*Ciliata, Holotricha*) in the course of division processes. Acta Protozool., 9, 283–298.

1973 — Golińska K. and Jerka-Dziadosz M. — The relationship between cell size and capacity for division in *Dileptus anser* and *Urostyla cristata*. Acta Protozool., 12, 1–21.

1974 — Effect of puromycin on regeneration processes in *Dileptus anatinus* Golińska, 1971. Acta Protozool., 12, 289–306.

1975 — Golińska K. and Bohatier J. — Action of Actinomycin D upon regenerative and divisional stomatogenesis in *Dileptus*. Acta Protozool., 14, 1–15.

1976 — Golińska K. and Kink J. — The regrowth of oral structures in *Dileptus cygnus* after partial excision. Acta Protozool., 15, 143–163.

1977 — Golińska K. and Kink J. — Proportional regulation of body form and cortical organelle pattern in the ciliate *Dileptus*. J. Cell Sci., 24, 11–29.

1977 — *See*: Jerka-Dziadosz, M. and Golińska K.

1978 — The course of *in situ* remodelling of injured mouthparts in *Dileptus* (*Ciliata, Gymnostomata*). Acta Protozool., 17, 47–67.

1979 — Regulation of ciliary pattern in *Dileptus*: sensory cilia and their conversion into locomotory cilia. J. Protozool., 26, 3, 81A, 255.

1979 — Assessment of cell proportions during regeneration of *Dileptus anser* (*Ciliata*). Wilhelm Roux' Archiv., 187, 307–321.

Grębecka Lucyna

1964 — Czarska (Grębecka) L. Role of the K^+ and CA^{2+} ions in the excitability of protozoan cell. Chemical and electric stimulation of contractile vacuoles. Acta Protozool., 2, 287–296.

1965 — Czarska (Grębecka) L. — Cytoplasmic streaming in *Paramecium caudatum* exposed to electric field. Acta Protozool., 3, 269–273.
1966 — Czarska (Grębecka) L. and Grębecki A. — Membrane folding and plasma-membrane ratio in the movement and shape transformation in *Amoeba proteus*. Acta Protozool., 4, 201–239.
1977 — Behaviour of anucleate anterior and posterior fragments of *Amoeba proteus*. Acta Protozool., 16, 87–105.
1977 — Changes of motory polarization in *Amoeba proteus* as induced by oil injections. Acta Protozool., 16, 107–120.
1978 — Frontal cap formation and origin of monotactic forms of *Amoeba proteus* under culture conditions. Acta Protozool., 17, 191–202.
1978 — Micrurgical experiments on the frontal cap of monotactic forms of *Amoeba proteus*. Acta Protozool., 17, 203–212.
1978 — Hrebenda B. and Grębecka L. — Ultrastructure of the frontal cap of monotactic form of *Amoeba proteus*. Cytobiologie, 17, 369–374.
1979 — Grębecka L. and Hrebenda B. — Topography of cortical layer in normally moving *Amoeba proteus*. Acta Protozool., 18, 493–502.

Grębecki Andrzej

1961 — Grębecki A. and Kuźnicki L. — Immobilization of *Paramecium caudatum* in the chloralhydrate solutions. Bull. Acad. Pol. Sci., Cl. II, 9, 459–462.
1962 — Phénoménes électrocinetiques dans le galvanotropisme de *Paramecium caudatum*. Bull. Biol. Fr. Belg., 96, 723–754.
1963 — Point isoélectrique superficiel et quelques reáctions locomotricec chez *Paramecium caudatum*. Protoplasma, 56, 80–88.
1963 — Electrobiologie d'ingestion des colorants par le cytostome de *Paramecium caudatum*. Protoplasma, 56, 89–98.
1963 — Grębecki A. and Kuźnicki L. — The influence of external pH on the toxicity of inorganic ions for *Paramecium caudatum*. Acta Protozool., 1, 157–164.
1963 — Rebroussement ciliaire et galvanotaxie chez *Paramecium caudatum*. Acta Protozool., 1, 99–112.
1963 — Galvanotaxie transversale et oblique chez les Ciliés. Acta Protozool., 1, 91–98.
1964 — Role des ions K^+ et Ca^{2+} dans l'excitabilite de la cellule protozaire. I. Equilibrement des ions antagonistes. Acta Protozool., 2, 69–79.
1964 — Modern lines in the study of amoeboid movement. Acta Protozool., 2, 379–402.
1964 — Calcium substitution in staining the cilia. Acta Protozool., 2, 375–377.
1965 — Gradient stomato-caudal d'excitabilité des Ciliés. Acta Protozool., 3, 79–101.
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.
1966 — *See*: Czarska (Grębecka) L. and Grębecki A.
1966 — *See*: Dryl S. and Grębecki A.
1967 — Grębecki A., Kuźnicki L. and Mikołajczyk E. — Some observations on the inversion of spiralling in *Paramecium caudatum*. Acta Protozool., 4, 383–388.
1967 — Grębecki A., Kuźnicki L. and Mikołajczyk E. — Right spiralling induced in *Paramecium* by Ni ions and the hydrodynamics of the spiral movement. Acta Protozool., 4, 389–408.

1968 — Grębecki A. and Mikołajczyk E. — Ciliary reversal and renormalization in *Paramecium caudatum* immobilized by Ni ions. Acta Protozool., 5, 297–304.
1975 — *See*: Grębecka L. and Grębecki A.
1977 — Non-axial cell frame movements and the locomotion of *Amoeba proteus*. Acta Protozool., 16, 53–85.
1977 — Grębecki A. and Nowakowska G. — On the mechanism of orientation of *Paramecium caudatum* in the gravity field. I. Influence of ciliary reversal and of external Ca deficiency on the geotactic behaviour. Acta Protozool., 16, 351–358.
1977 — *See*: Nowakowska G. and Grębecki A.
1978 — Grębecki A. and Grębecka L. — Morphodynamic types of *Amoeba proteus* a terminological proposal. Protistologica, 14, 349–358.
1978 — Grębecki A., Kalinina L. and Grębecka L. — Responses to light-shade difference in enucleate and polynucleate specimens of *Amoeba proteus*. Cytobiologie, 17, 343–353.

Hrebenda Barbara

1973 — The role of external calcium in motile phenomena of *Amoeba proteus*. Acta Protozool., 12, 107–111.
1974 — *See*: Rinaldi R. A., Opas M. and Hrebenda B.
1976 — Minimal requirements of external calcium for motility of *Amoeba proteus*. Acta Protozool., 12, 107–111.
1977 — *See*: Sobota A., Hrebenda B. and Przełęcka A.
1978 — Hrebenda B. and Grębecka L. — Ultrastructural of the frontal cap of monotactic form of *Amoeba proteus*. Cytobiologie, 17, 62–72.
1979 — *See*: Grębecka L. and Hrebenda B.

Jerka-Dziadosz Maria

1963 — Morphogenesis in division and regeneration of *Urostyla grandis* Ehrbg. Acta Protozool., 1, 43–54.
1964 — Localization of the organization area in course of regeneration of *Urostyla grandis* Ehrbg. Acta Protozool., 2, 129–136.
1967 — Traumatic disturbance of cell division and regeneration of fragments derived from dividing individuals *Urostyla*. Acta Protozool., 5, 59–79.
1968 — Study on resorption of ciliature in *Urostyla* (*Hypotricha*). Acta Protozool., 5, 359–368.
1969 — Jerka-Dziadosz M. and Frankel J. — An analysis of formation of ciliary primordia in the hypotrich ciliate *Urostyla weissei*. J. Protozool., 16, 612–637.
1970 — Jerka-Dziadosz M. and Frankel J. — The control of DNA synthesis in macronuclei and micronuclei of a hypotrich ciliate: a comparison of normal and regenerating cells. J. Exp. Zool., 173, 1–22.
1972 — Cortical development in *Urostyla*. I. Comparative study on morphogenesis in *U.cristata* and *U.grandis*. Acta Protozool., 10, 73–100.
1973 — *See*: Golińska K. and Jerka-Dziadosz M.
1974 — Cortical development in *Urostyla*. II. The role of positional information and performed structures in formation of cortical pattern. Acta Protozool., 12, 239–274.
1976 — The proportional regulation of cortical structures in a hypotricha ciliate *Paraurostyla waissei*. J. Exp. Zool., 195, 1–14.

1977 — Jerka-Dziadosz M. and Golińska K. — Regulation of ciliary pattern in ciliates. J. Protozool., 24, 19–26.
1979 — Jerka-Dziadosz M. and Frankel J. — A mutant of *Tetrahymena* with a partial mirror-image duplication of cell surface pattern. I. Analysis of the phenotype. J. Embryol. Exp. Morph., 49, 167–202.

Kaczanowska Janina

1969 — Kaczanowska J. and Kowalska D. — Studies on topography of the cortical organelles of *Chilodonella cucullulus* (O.F.M.). I. The cortical organelles and intraclonal dimorphism. Acta Protozool., 7, 1–15.
1971 — Topography of cortical organelles in early dividers of *Chilodonella cucullulus* (O.F.M.). Acta Protozool., 8, 231–250.
1971 — Studies on topography of the cortical organelles of *Chilodonella cucullulus* (O.F.M.). III. Morphogenetic movements regional multiplication of kinetosomes and cytokinesis in normal dividers, and after phenethyl alcohol treatment. Acta Protozool., 9, 83–104.
1974 — The pattern of morphogenetic control in *Chilodonella cucullulus*. J. Exp. Zool., 187, 47–62.
1975 — Shape and pattern regulation in regenerants of *Chilodonella cucullulus* (O.F.M.). Acta Protozool., 13, 343–360.
1976 — Termosentivity of pattern formation in a ciliate *Chilodonella cucullulus*. J. Exp. Zool., 198, 135–148.
1979 — Physiological dissection of various effects of ruthenium red dye on *Paramecium* cell. Experientia, 35, 1062–1064.

Kaczanowski Andrzej

1963 — Morphology and morphogenesis of the sucker in *Hysterocineta paludinarum* Diesing (*Ciliata, Thigmotricha*) as observed in living material. Acta Protozool., 1, 395–401.
1965 — About a presumable phylogenetic link between *Mesnilella* (*Astomata*) and *Conchostoma longissimum* Fauré-Fremiet (*Trichostomata*). Acta Protozool., 3, 225–232.
1970 — Morphological studies on opalinids. I. Staining and fragmentation of the pellicle of *Opalina ranarum*. Acta Protozool., 7, 205–209.
1971 — *Opalina ranarum* Purkinje et Valentin: meiosis and dimorphism of nuclear behaviour during meiosis. Acta Protozool., 9, 105–106.
1972 — Kaczanowski A., Gołembiewska M. and Mazur M. — Studies on encystation and excystation in *Opalina ranarum*. Acta Protozool., 10, 185–193.
1973 — Morphological studies on opalinids. II. Cortical patterns in *Opalina ranarum*. Acta Protozool., 12, 29–52.
1975 — A single-gene-dependent abnormality of adoral membranelles in *Tetrahymena pyriformis*, species 1. Genetics, 81, 63–639.
1976 — An analysis of mp gene affected morphogenesis in *Tetrahymena pyriformis*, syngen 1 (species 1) Ciliates. J. Exp. Zool., 196, 215–230.
1978 — Gradients of proliferation of ciliary basal bodies and the determination of the position of the oral primordium in *Tetrahymena*. J. Exp. Zool., 204, 417–429.

Kasprzak Witold

1961 — Biological characteristics of the strains of *Entamoeba histolytica* from the carriers in Poznań Palatinate. Acta Parasit. Polon., 9, 211–230.

1968 — The effect of some biological factors on the course of experimental amoebiasis. Acta Parasit. Polon., 15, 333–354.
1972 — Kasprzak W. and Mazur T. — Free-living amoebae isolated from water frequented by people in the vivinity of Poznań, Poland. Experimental studies in mice on the pathogenicity of the isolates. Z. Tropenmed. Parasitol., 23, 391–398.
1973 — Kasprzak W., Myjak P. and Mazur T. — Virulence of indigenous strains of *Entamoeba histolytica* in experimental amoebiasis of rats. Bull. Inst. Mar. Med., 24, 305–316.
1974 — Kasprzak W., Mazur T. and Rucka A. — Studies on some pathogenic strains of free-living amoebae isolated from lakes in Poland. Ann. Soc. Belg. méd. trop., 54, 351–357.
1976 — Kasprzak W. and Mazur T. — The effect of thermic pollution of water on the distribution of pathogenic strains of *Naegleria*. Wiad. Parazyt., 22, 457–459.
1977 — [Hadaś E.], Kasprzak W. and Mazur T. — Electrophoretic characterization of small free-living amoebae. Z. Tropenmed. Parasitol., 28, 35–43.

Kazubski Stanisław

1958 — *Thigmocoma acuminata* gen. nov. sp. nov. (*Thigmotricha—Thigmocomidae* fam. nov.) a parasite of the renal organ of *Schistophallus orientalis* Cless. (*Pulmonata—Zonitidae*). Bull. Acad. Pol. Sci., Cl. II, 6, 4, 167–172.
1958 — *Trichia lubomirski* Ślós. (*Helicidae*), a new host of *Tetrahymena limacis* (Warren, 1932), Kozloff, 1946, (*Ciliata*) and *Zonitoides nitidus* Müll. (*Zonitidae*), a new host of *T. rostrata* (Kahl, 1926) Corliss, 1952 in Poland. Bull. Acad. Pol. Sci., Cl. II, 6, 6, 247–252.
1963 — Studies on the parasitic ciliate *Thigmocoma acuminata* Kazubski (*Thigmotricha—Thigmocomidae*). Acta Protozool., 1, 237–278.
1964 — Contribution to the study of the fauna of parasitic ciliates in the land snail *Poiretia algira* (Brug.) from Yugoslavia. Acta Protozool., 2, 159–162.
1967 — Study on the growth of skeletal elements in *Trichodina pediculus* Ehrbg. Acta Protozool., 5, 37–48.
1968 — Kazubski S. L. and Migała K. — *Urceolariidae* from breeding carp — *Cyprinus carpio* L. in Żabieniec and remarks on the seasonal variability of trichodinids. Acta Protozool., 6, 137–160.
1968 — Seasonal variability of *Thigmocoma acuminata* Kazubski, 1958 (*Thigmotricha*). Acta Protozool., 6, 353–355.
1971 — Morphological variability of *Semitrichodina sphaeronuclea* (Lom, 1956). Acta Protozool., 8, 251–260.
1972 — Kazubski S. L. and Migała K. — Studies on the distinctness of *Chilodonella cyprini* (Moroff) and *Ch. hexasticha* (Kiernik) (*Chlamydodontidae, Gymnostomatida*) ciliate parasite of fishes. Acta Protozool., 13, 9–40.
1976 — On the variability of a parasitic ciliate *Semitrichodina sphaeronuclea* (Lom) (*Urceolariidae*) according to the altitude of its habitats above sea level. Acta Protozool., 15, 29–34.
1979 — *Trichodina vesicularum* Fauré-Fremiet, 1943 and *T. faurefremieti* nom.

nov. (*Ciliata, Peritrichida*) — parasites of newts of the genus *Triturus*. Acta Protozool., 18, 371–384.

1979 — Morphological variability of *Trichodina vesicularum* Fauré-Fremiet and *T. faurefremieti* Kazubski, parasites of newts from Poland and France. Acta Protozool., 18, 385–400.

Kink Jolanta

1972 — Observations on morphology and oral morphogenesis during regeneration of ciliate *Lacrymaria olor* (O.F.M.) Acta Protozool., 10, 205–214.

1973 — The organization of fibrillar structures in the trophic and encysted *Dileptus visscheri* (*Ciliata, Rhabdophorina*). Acta Protozool., 12, 173–194.

1976 — A localized region of basal bodies proliferation in growing cells of *Dileptus visscheri Ciliata Gymnostomatida*. J. Cell. Sci., 20, 115–133.

Komala Zofia

1975 — The effect of some pesticides on *Paramecium aurelia*. Differing in genetic affinity. Folia Biol. (Kraków), 23, 231–243.

1975 — The effect of X-rays on strains and syngens of *Paramecium aurelia*. Folia Biol., (Kraków), 23, 341–423.

Korohoda Włodzimierz

1968 — Korohoda W., Forrester J. A., Moreman G. and Ambrose E. J. — Size changes in isolated nuclei of *Amoeba proteus* on treatment with polyionic substances. Nature, 217, 615–617.

1970 — Korohoda W. and Kurowska A. — Quantitative estimations of the thresholds of electrotactic responses in *Amoeba proteus*. Acta Protozool., 7, 375–382.

1973 — *See*: Fabczak S., Walczak T. and Korohoda W.

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

1977 — Experimental indication of locomotion in enucleated fragments of *Amoeba proteus* and its bearing on the theories of ameboid movement. Cytobiologie, 14, 338–349.

Kościuszko Halina

1965 — Karyologic and genetic investigations in syngen 1 of *Paramecium aurelia*. Folia Biol., (Kraków), 13, 339–368.

1971 — The micronuclear content of DNA in six strains of *Paramecium aurelia*, syngen 1. Folia Biol., (Kraków), 19, 363–370.

1976 — Estimation of allelic immobilization antigens in syngen 9 of *Paramecium aurelia*. III. Serotype G variation. Folia Biol., (Kraków), 24, 13–39.

1976 — Estimation of allelic immobilization antigens in syngen 9 of *Paramecium aurelia*. I. Microtechnique with complement fixation, in conjunction with other methods. Folia Biol., (Kraków), 24, 3–12.

Krawczyńska Wanda

1978 — Krawczyńska W., Rowiński J., Przełęcka A. — Morphometric analysis of macronuclei and macronuclear fragments in autogamous cultures of *Paramecium aurelia*. Acta histochem., 63, 153–161.

1979 — Ultrastructure of isolated *Paramecium aurelia* macronuclei. Acta Protozool., 18, 231–236.

Kuźnicki Leszek

1961 — *See*: Grębecki A. and Kuźnicki L.

1963 — Recovery in *Paramecium caudatum* immobilized by chloralhydrate treatment. Acta Protozool., 1, 177–185.

1963 — Reversible immobilization of *Paramecium caudatum* evoked by nickel ions. Acta Protozool., 1, 301–312.

1963 — *See*: Grębecki A. and Kuźnicki L.

1966 — 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.

1966 — Ciliary reversal in *Paramecium caudatum* in relation to external pH. Acta Protozool., 4, 257–261.

1966 — Kuźnicki L. and Sikora J. — Inversion of spiralling of *Paramecium aurelia* after homologous antiserum treatment. Acta Protozool., 4, 263–268.

1967 — *See*: Grębecki A., Kuźnicki L. and Mikołajczyk E.

1967 — *See*: Grębecki A., Kuźnicki L. and Mikołajczyk E.

1968 — Behavior of *Paramecium* in gravity fields. I. Sinking of immobilized specimens. Acta Protozool., 6, 109–118.

1970 — Mechanisms of the motor responses of *Paramecium*. Acta Protozool., 8, 83–118.

1970 — Kuźnicki L., Jahn T. L. and Fonseca J. R. — Helical nature of the ciliary beat of *Paramecium multimicronucleatum*. J. Protozool., 17, 16–24.

1971 — Kuźnicki L. and Sikora J. — Cytoplasmic streaming within *Paramecium aurelia*. I. Movements of crystals after immobilization by antiserum. Acta Protozool., 8, 439–445.

1972 — Kuźnicki L. and Sikora J. — The hypothesis of inverse relation between ciliary activity and cyclosis in *Paramecium*. Acta Protozool., 11, 242–250.

1972 — Kuźnicki L., Sikora J. and Fabczak S. — Cytoplasmic streaming within *Paramecium aurelia*. II. Cinematographic analysis of the course and reversible cessation of cyclosis. Acta Protozool., 11, 237–242.

1973 — Kuźnicki L. and Sikora J. — Cytoplasmic streaming within *Paramecium aurelia*. III. The effect of temperature of flow velocity. Acta Protozool., 12, 59–66.

1976 — *See*: Sikora J. and Kuźnicki L.

1979 — Kuźnicki L. and Kuźnicki J. — Speculations on evolution of mechanisms regulating actin and myosin interaction. Acta Protozool., 18, 97–107.

1979 — [Kuźnicki J.], Kuźnicki L. and Drabikowski W. — Ca^{2+}-binding modular protein in *Protozoa* and *Myxomycete*. Cell Biol. Int. Rep., vol. 3, 1, 17–23.

Kwiatkowska-Grabacka Elżbieta

1965 — Microfauna of the bottom fish ponds in Gołysz. Acta Hydrobiol., 7, 317–328.

1971 — Grabacka E. — Ciliata of the bottom of reaning fishponds in the Gołysz Complex. Acta Hydrobiol., 13, 5–28.
1973 — Protozoans in ponds filled with sugar factory wastes. Acta Hydrobiol., 15, 97–111.
1977 — The influence of beet sugar factory wastes on the bottom microfauna in fish ponds. Acta Hydrobiol., 19, 373–387.

Lipa Jerzy J.

1957 — Observations on development and pathogenicity of the parasite of *Aporia crataegi* L. (*Lepidoptera*) *Nosema aporiae* n. sp. Acta Parasitol. Pol., 5, 559–584.
1962 — Lipa J. J. and Steinhaus E. A. — Further report on identification of protozoa pathogenic for insects. Acta Parasitol. Pol., 10, 165–175.
1967 — Studies on gregarines (*Gregarinomorpha*) of arthropods in Poland. Acta Protozool., 5, 97–180.
1968 — *Nosema leptinotarsae* sp. n., a microsporidian parasite of the Colorado potato beetle. (*Leptinotarsa decemlineata*). J. Invertebr. Pathol., 10, 111–115.
1968 — On gregarine parasites of *Coccinellidae* in California, USA. Acta Protozool., 4, 263–272.
1968 — Some observations on *Nosema heliothidis* Lutz et Splendore, a microsporidian parasite of *Heliothis zea* (Boddie), (*Lepidoptera, Noctuidae*). Acta Protozool., 6, 273–280.
1976 — Microorganisms in adults of several noctuid species (*Noctuidae*: *Lepidoptera*) caught in the light trap during 1973–1975. Bull. Acad. Pol., Ser. Sci. Biol., Cl. V., 24, 783–787.
1977 — Infection of *Notocellia uddmanniana* L. (*Lepidoptera*: *Tortricidae*) by the microsporidian *Nosema carpocapsae* Paillot. Acta Protozool., 16, 201–205.
1977 — *Thelohania ostriniae* n. sp. A new microsporidian parasite of the European corn borer *Ostrinia nubilalis* Hbn. (*Lepidoptera, Pyralidae*). Acta Protozool., 16, 151–155.
1979 — Lipa J. J. and Simchuk P. — *Chrysomela menthastri* Suffr. as a host for *Gregarina minieri* (Schneider) and *Gregarina crenata* (Bhatia et Setna). Bull. Acad. Pol. Sci., Cl. II, 27/2, 105–108.

Michajłow Włodzimierz

1968 — *Euglenoidina* (*Flagellata*) — parasites of *Cyclopidae* (*Copepoda*). Acta Protozool., 5, 181–217.
1971 — Michajłow W. and Wita I. — *Embryocola cordiformis* sp. n. (*Euglenoidina*) — parasite of *Copepoda* from the environs of Warsaw. Bull. Acad. Pol. Sci. Ser. Biol., 19, 417–419.
1972 — *Euglenoidina* parasitic in *Copepoda*. An outline monograph. PWN, 1–224.
1976 — Michajłow W. and Wita L. — *Embryocola diflagellatus* sp. n. (*Euglenoidina*) parasite of *Copepoda* from the environs of Warsaw. Bull. Acad. Pol. Sci., Ser. Biol., 24, 10, 615–618.
1976 — Michajłow W. and Wita I. — *Paradistigmoides kenyensis* g. n., sp. n. and *Naupliicola kenyensis* sp. n. (*Euglenoidina*) — parasites of *Copepoda* from Kenya. Bull. Acad. Pol. Sci., Ser. Sci. Biol., 24, 283–288.

Michejda Jan

1977 — [Hryniewiecka L.] and Michejda J. W. — Configuration of *Acanthamoeba castellanii* Neff in negative staining. Bull. Soc. Amis. Sci. Lett. Poznań, Ser. D-17, 147–155.

1977 — [Hryniewiecka L.] and Michejda J. W. — Configuration of *Acanthamoeba castellanii* mitochondria in various energetical states. Bull. Soc. Amis. Sci. Lett. Poznań, Ser. D-17, 129–145.

1978 — [Hryniewiecka L.], Jenek J. and Michejda J. W. — Cyanide resistance in soil amoeba *Acanthamoeba castellanii*. In: Plant Mitochondria (G. Ducet, C. Lance eds) Elsevier Press, Amsterdam.

1979 — [Hryniewiecka L.], Domka-Popek A. and Michejda J. — Stimulation of respiration of *Acanthamoeba castellanii* mitochondria by calcium ions. Bull. Soc. Amis. Sci. Lett. Poznań, Ser. D-19, 13–23.

Migała Kazimierz

1968 — *See*: Kazubski S. L. and Migała K.

1971 — Studies on natural populations of parasitic protozoa on *Cyprinus carpio* L. in pond culture. Carps in the first year of life. Acta Protozool., 8, 309–339.

1972 — Migała K. and Kazubski S. L. — Occurrence of non-specific ciliates on carps (*Cyprinus carpio* L.) in winter ponds. Acta Protozool., 9, 329–337.

1972 — *See*: Kazubski S. L. and Migała K.

1972 — Migała K. and Grygierek E. — On the occurrence of *Trichodina domerguei* subsp. *megamicronucleata* Haider (*Ciliata, Peritricha*) on *Eudiaptomus zachariasi* Poppe (*Calanoida*). Acta Protozool., 10, 269–274.

1974 — *See:* Kazubski S. L. and Migała K.

Mikołajczyk Ewa

1967 — *See*: Grębecki A., Kuźnicki L. and Mikołajczyk E.

1968 — *See*: Grębecki A. and Mikołajczyk E.

1975 — Mikołajczyk E. and Diehn B. — The effect of potassium iodide on photophobic responses in *Euglena*: Evidence for two photoreceptor pigments. J. Photochem. Photobiol., 22, 269–271.

1978 — Mikołajczyk E. and Diehn B. — Morphological alterations in *Euglena gracilis* induced by treatment with CTAB (Cethyltrimethyl ammonium bromide) and Triton X-100, correlations with effects on photophobic behavioural responses. J. Protozool., 25, 461–470.

1976 — Mikołajczyk E. and Diehn B. — Light induced body movement of *Euglena gracilis* coupled to flagellar photophobic responses by mechanical stimulation. J. Protozool., 25, 144–147.

1978 — *See*: Grębecki A. and Mikołajczyk E.

1979 — Mikołajczyk E. and Diehn B. — Mechanosensory responses and mechanoreception in *Euglena gracilis*. Acta Protozool., 18, 591–602.

Myjak Przemysław

1971 — Diagnostics of *Entamoeba histolytica* by means of cultures on solid media. Biul. Inst. Med. Morsk. Trop., 22, 165–171.

1972 — The use of solid-medium cultures in chemotherapeutic experiments *in vitro* with *Entamoeba histolytica*. Biul. Inst. Med. Morsk. Trop., 23, 75–83.

1974 — The use of solid medium cultures for the removal of bacteria and

fungi from *Entamoeba histolytica* strains. Biul. Inst. Med. Morsk. Trop., 25, 113–120.

1979 — Myjak P., Mrozińska W., Kudrewicz L. and Zwierz Cz. — The occurrence of antibodies *Entamoeba histolytica* in persons returning from the tropics and in those who did not leave Poland. Biul. Inst. Med. Morsk. Trop., 30, 45–58.

Opas Michał

1974 — *See*: Rinaldi R., Opas M. and Hrebenda B.

1975 — *See*: Rinaldi R., Opas M. and Hrebenda B.

1976 — Opas M. and Rinaldi R. — Ca^{++} controlled contraction-relaxation cycle in glycerinated ameboid cells. Protoplazma, 90, 393–397.

1976 — *See*: Rinaldi R. and Opas M.

1976 — Course of glycerination of *Amoeba proteus* and contraction of glycerinated models. Acta Protozool., 15, 485–499.

1978 — Interference reflection microscopy of adhesion of *Amoeba proteus*. J. Microsc., 122, 215–221.

1978 — Holographic microscopy of glycerination of *Amoeba proteus*. J. Microsc., 112, 301–305.

1978 — Opas M., Hrebenda B. and Tołłoczko B. — Contractility of glycerol-extracted nuclei of *Amoeba proteus*. Acta Protozool., 17, 369–374.

Pado Ryszard

1965 — Mutual relation of protozoans and symbiotic algae in *Paramecium bursaria*. I. The influence of light of the growth of symbionts. Folia Biol., (Kraków), 13, 178–182.

1967 — Mutual relation of protozoans and symbiotic algae in *Paramecium bursaria*. II. Photosynthesis. Acta Soc. Bot. Pol., 36, 97–108.

1972 — Spectral activity of light and phototaxis in *Paramecium bursaria*. Acta Protozool., 11, 387–393.

Pożaryska Krystyna

1957 — Lagenidae du Cretace Superieur en Pologne. Paleontol. Pol., 8, 1–190.

1965 — Foraminifera and biostratigraphy of the Danian and Montain in Poland. Paleontol. Pol., 14, 1–156.

1968 — [Szczechura J.] and Pożaryska K. — Foraminifera from the Paleocene of Poland their ecological biostratigraphical meaning. Paleontol. Pol., 20, 1–107.

1968 — Pożaryska K. and Brochwicz-Lewiński W. — The nature and origin of Mesozoic and Cenozoic marine faunal provinces. Mitt. Geol. Pal. Univ. Hamburg, 44, 207–216.

1968 — Pożaryska K., Neumann M. and Vachard D. — Remarques sur les microfacies du Devonien de Lublin (Pologne). Rev. Microp., 18, 38–52, Paris .

Przełęcka Aleksandra

1973 — *See*: Wyroba E. and Przełęcka A.

1977 — Przełęcka A., Sobota A. and Brutkowska M. — A characteristic pattern of Ca-dependent deposits in cilia of *Tetrahymena pyriformis* GL. Bull. Acad. Pol. Sci., Ser. Biol., Cl. II, 25, 183–186.

1977 — *See*: Hrebenda B., Sobota A. and Przełęcka A.
1977 — *See*: Sobota A., Hrebenda B. and Przełęcka A.
1978 — *See*: Sobota A., Przełęcka A. and Janossy A. G. S.

Przyboś Ewa
1975 — Genetic studies of *Paramecium jenningsi* strains (Diller, Earl, 1958). Folia Biol., (Kraków), 23, 425–471.

Radzikowski Stefan
1965 — Changes in the heterometric macronucleus in division of *Chilodonella cucullulus* (Müller). Acta Protozool., 3, 233–238.
1966 — Study on morphology, division and postconjugation morphogenesis in *Chilodonella cucullulus*. (O. F. Müller). Acta Protozool., 4, 89–96.
1973 — Elimination of chromatin from the micronucleus of *Chilodonella cucullulus* OFM during the 1st meiotic division. Acta Protozool., 12, 105–109.
1973 — Die Entwicklung synthese während der Konjugation von *Chilodonella cucullulus* OFM. Arch. Protistenk., 115, 419–433.
1973 — Ultrastructure of the nuclear apparatus in *Chilodonella cucullulus* OFM with observations on number and size of nucleoli during interphase. Acta Protozool., 12, 23–28.
1976 — DNA and RNA synthesis in the nuclear apparatus of *Chilodonella cucullulus* OFM. Acta Protozool., 15, 47–56.
1979 — Asynchronous replication of polyetene chromosome segments of the new macronucleus analoge in *Chilodonella cucullulus* OFM. Protistologica, 15, 521–526.

Rinaldi Robert A.
1974 — Rinaldi R. A., Opas M. and Hrebenda B. — Contractility of glycerinated *Amoeba proteus* and *Chaos chaos*. J. Protozool., 22, 286–292.
1975 — Rinaldi R. and Hrebenda B. — Oriented thick and thin filaments in *Amoeba proteus*. J. Cell. Biol., 66, 193–198.
1976 — *See*: Opas M. and Rinaldi R.
1976 — *See*: Opas M. and Rinaldi R.

Rostkowska Julia
1963 — Effect of chemical agents on the survival of *Balantidium coli* (Malmsten). Acta Protozool., 1, 421–433.
1970 — Studies on the infective stage of *Balantidium coli* (Malmsten) for hamsters. Acta Protozool., 7, 269–276.
1970 — Biocoenotic interrelations between *Balantidium coli* (Malmsten) and the bacterial flora of the alimentary tract. Acta Parasitol. Polon., 18, 377–392.
1978 — *See*: Balcerzak Cz. and Rostkowska J.

Sikora Jerzy
1966 — Immobilization by homologous antiserum and antigenic transformation

in *Paramecium aurelia* in relation to the ionic composition of medium. Acta Protozool., 4, 143–154.
1966 — *See*: Kuźnicki L. and Sikora J.
1971 — *See*: Kuźnicki L. and Sikora J.
1972 — *See*: Kuźnicki L. and Sikora J.
1972 — *See*: Kuźnicki L., Sikora J. and Fabczak S.
1973 — *See*: Kuźnicki L. and Sikora J.
1975 — A new method of locomotion arresting in some ciliates without ciliary immobilization. Acta Protozool., 13, 421–424.
1976 — Sikora J. and Kuźnicki L. — Cytoplasmic streaming within *Paramecium aurelia*. IV. Cyclosis during binary fission and conjugation. Acta Protozool., 15, 173–178.
1978 — Sikora J. and Wasik A. — Cytoplasmic streaming within Ni^{2+} immobilized *Paramecium aurelia*. Acta Protozool., 17, 389–397.
1979 — Sikora J., Wasik A. and Baranowski Z. — The estimation of velocity distribution profile of *Paramecium* cytoplasmic streaming. Europ. J. Cell. Biol., 19, 184–188.

Skoczylas Bogna

1963 — Skoczylas B., Panusz H. and Gross M. — Isolation of macronuclei from *Paramecium caudatum*. The role of calcium ions. Acta Protozool., 1, 411–420.
1966 — Gross M., Skoczylas B. and Turski W. — Purification and some properties of ribonucleases from *Paramecium aurelia*. Acta Protozool., 4, 59–66.
1972 — Deoxyribonuclease in *Paramecium aurelia*, syngen 4, strain 51. Acta Protozool., 10, 215–224.
1975 — Skoczylas B. and Soldo A. T. — Separation and purification of macronuclei from macronuclear fragments and micronuclei in the ciliate *Paramecium aurelia*. Expl. Cell. Res., 90, 143–152.
1975 — Skoczylas B. and Van Wagtendonk W. J. — A maintenance medium for the axenic culture of *Paramecium aurelia*. Acta Protozool., 14, 379–383.

Sobota Andrzej

1977 — Sobota A., Hrebenda B. and Przełęcka A. — Formation of calcium-dependent deposits at the plasma membrane of *Acanthamoeba castellanii*. Cytobiologie, 15, 259–268.
1977 — *See*: Hrebenda B., Sobota A. and Przełęcka A.
1977 — *See*: Przełęcka A., Sobota A. and Brutkowska M.
1978 — Sobota A., Przełęcka A. and Janossy A. G. S. — X-ray microanalysis at the plasma membrane of *Acanthamoeba castellanii*. Cytobiologie, 17, 464–469.

Sołtyńska Maryla

1975 — Morphology and fine structure of *Chilodonella cucullulus* (O.F.M.). Cortex and cytopharyngeal apparatus. Acta Protozool., 9, 49–82.

Tołłoczko Barbara

1975 — Endocytosis in *Paramecium*. I. Effect of tripsine and pronase. Acta Protozool., 14, 313–320.

1976 — Endocytosis in *Paramecium*. II. Effect of lysozome and neuraminidase. Acta Protozool., 15, 359–366.
1977 — Endocytosis in *Paramecium*. III. Effect of cytochalasin B and colchicine. Acta Protozool., 16, 185–193.
1978 — *See*: Opas M., Hrebenda B. and Tołłoczko B.

Totwen-Nowakowska Irena

1964 — Doublets in a clone of *Stylonychia mytilus* (O.F.M.). Acta Protozool., 2, 137–146.
1965 — Doublets of *Stylonychia mytilus* (O.F.M.) evoked by action of thermic shocks. Acta Protozool., 3, 355–361.
1973 — Conditions of stability of the double system in the post-traumatic regeneration of doublets of *Stylonychia mytilus*. Acta Protozool., 12, 53–68.
1977 — *See*: Dryl S. and Totwen-Nowakowska I.

Wartoń Andrzej

1972 — Wartoń A. and Kallinikova V. — Citochimičeskoe spravnienie niekotoryh vidov trypanosom v sviazi so sposobnostju k diskinetoplastii. I. *Trypanosoma cruzi* i *T. equiperdum*. Cytologia, 14, 647–654.
1974 — Wartoń A. and Kallinikova V. — Cytochemistry of some *Trypanosoma* species as related to their capacity for diskinetoplastia. II. *Trypanosoma lewisi* and *T. evansi*. Acta Protozool., 12, 355–362.
1974 — Wartoń A. and Kallinikova V. — Cytochemical study of diskinetoplastic forms of *Trypanosoma equiperdum* and *T. evansi*. Acta Protozool., 13, 225–233.
1975 — Wartoń A. and Modlińska M. — The effect of acriflavine on the dynamics of the trypanosome population size and the formation of diskinetoplastic forms in the host blood. Acta Parasitol. Pol., 23, 127–133.
1979 — Wartoń A. and Honigberg B. — Structure of trichomonads as revealed by scanning electron microscopy. J. Protozool., 26, 56–62.

Wita Irena

1971 — *Naupliicola ergasili* sp. n. — Parasite of *Ergasilus sieboldi* (Nordmann) larvae from Poland. Bull. Acad. Pol. Sci., Ser. Sci. Biol. 19, 351–353.
1972 — *Parastasiella ergasili* sp. n. — Parasite of *Ergasilus sieboldi* (Nordmann) Eggs in Poland. Bull. Acad. Pol. Sci., Ser. Sci. Biol., 20, 133–137.
1972 — *See*: Michajłow W. and Wita I.
1976 — *See*: Michajłow W. and Wita I.
1976 — *See*: Michajłow W. and Wita I.

Wolska Maria

1966 — Study on the family *Blepharocorythidae* Hsiung. I. Preliminary remarks. Acta Protozool., 4, 97–104.
1967 — Study on the family *Blepharocorythidae* Hsiung. II. *Charonina ventriculi* (Jameson). Acta Protozool., 4, 279–284.
1967 — Study on the family *Blepharocorythidae* Hsiung. III. *Raabena bella* gen. n., sp. n. from the intestine of the Indian elephant. Acta Protozool., 4, 285–290.

1968 — Study on the family *Blepharocorythidae* Hsiung. IV. *Paraabena dentata* gen. n., sp. n. from the intestine of Indian elephant. Acta Protozool., 5, 219–224.

1971 — Studies on the family *Blepharocorythidae* Hsiung. V. A review of genera and species. Acta Protozool., 9, 23–40.

1971 — Studies on the family *Blepharocorythidae* Hsiung. VI. Phylogenesis of the family and the description of the new genus. *Circodinium* gen. n. with the species *C. minimum* (Gassovsky, 1918). Acta Protozool., 9, 171–194.

1978 — *Tripalmaria dogieli* Gass., 1928 (*Ciliata, Entodiniomorphida*). Structure and ultrastructure. Part II. Electron-microscope investigations. Acta Protozool., 17, 21–30.

1978 — *Tripalmaria dogieli* Gass., 1928 (*Ciliata, Entodiniomorphida*). Structure and ultrastructure. Part I. Light-microscope investigations. Acta Protozool., 17, 13–20.

1978 — Light and electron microscope studies on *Ochoterenaia appendiculata* Chavarria (*Ciliata, Blepharocorythina*). Acta Protozool., 17, 483–492.

Wyroba Elżbieta

1973 — Wyroba E. and Przełęcka A. — Studies on the surface coat of *Paramecium aurelia*. I. Ruthenium red staining and enzyme treatment. Z. Zellforsch., 143, 343–353.

1977 — Studies on the surface coat of *Paramecium aurelia*. II. Relationship to the immobilization antigen. Cell Tiss. Res., 181, 245–253.

1978 — Cell multiplication following partial enzymatic removal of surface coat. Cytobiologie, 17, 412–420.

1978 — Wyroba E. and Brutkowska M. — Restoration of phagocytic activity of *Paramecium tetraurelia* suppressed by previous digestion of surface coat. Acta Protozool., 17, 515–524.

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Symposium E

Ultrastructure and Molecular Background of Motility

Introduction

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Motive force generation for motility in *Protozoa*, as in other non-muscle eukaryotic cells, represents a modern research area of general cell biology. Essentially, the chemo-mechanical energy transformation is brought about by three main protein systems: (1) cytoplasmic actin and myosin, (2) tubulin and dynein, (3) Ca^{++}-binding proteins (only in certain ciliates).

The tubulin-dynein system in cilia and flagellae is rather well known. Recent progress in this field will be presented by P. Satir and M. Sleigh. In the case of the cytoplasmic microtubules, however, it is an open question whether they are able to perform chemo-mechanical energy transformations for motility phenomena or whether they represent "only" cytoskeletal elements. To date, the search for a corresponding ATP-ase (like dynein in cilia and flagellae) has not been successful.

The cytoplasmic actomyosin system ("microfilaments") appears as a random network within the groundplasm of all eukaryotic cells. The main structural element of the network is F-actin; binding

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sites are actin-myosin connections and "actin-binding proteins". The network is highly dynamic in nature and is able to perform cytoskeletal functions as well as chemo-mechanical energy-transformations. The actin filaments can aggregate in parallel arrays containing oligomeric myosin ("actomyosin fibrils", "stress fibrils"). Both the network and the fibrils are able to perform cytoskeletal and contractile functions. Moreover, the network is responsible for rapid visconsity changes of the cytoplasm, e.g., in the process of ameboid movement (ecto ⇌ endoplasmic transformation).

What is the Molecular Contraction Mechanism of Cytoplasmic Actomyosin?

Isolated actomyosin fibrils as well as "synthetic" actomyosin fibrils undergo isotonic contractions under appropriate conditions. The contraction process is accompanied by a transformation of the previously parallel-arranged actin chains into a dense network structure of filaments. The parallel arrangement of F-actin, however, is not a precondition for contraction: A random network of F-actin and oligomeric myosin performs volume contractions designated as "superprecipitation of actomyosin" in biochemical investigations. This contraction of a random network of actomyosin leads as well to a condensation of the network structure. Irrespective of the previous arrangement of the actin chains, i.e., parallel or non-parallel arrangement, an isotonic contraction always results in a condensed network of actin (and myosin); moreover, the thickness of F-actin does not appear to undergo changes. This observation is at least compatible with the assumption that cytoplasmic actomyosin contracts by a sliding mechanism comparable to cross-striated muscle.

Interpretation of the Fibrillar State

Actomyosin fibrils of light microscopic dimensions, e.g., in *Physarum polycephalum* and in tissue culture cells ("stress fibrils"), represent the isometrically contracted state, i.e., they perform a certain tension along their longitudinal axis and thus between their points of insertion (plasmalemma and/or ramification points of other fibrils). For an analysis of the contraction–relaxation cycle and thus for the molecular contraction mechanism it is important to note that all other states of the cycle are non-fibrillar, i.e., they show no parallel arrangement of F-actin (isometric relaxation, isotonic relaxation and contraction).

Transformations of Actin

The analysis of the contraction mechanism of cytoplasmic actomyosin must take into account changes of the polymer status of actin, i.e., variations in the length of the actin chain during the contraction cycle. In recent years it became apparent that not only in ameboid moving cells, but probably also in all eukaryotic cells an "actin-equilibrium" is maintained in the sense that a certain amount of this protein is filamentous, whereas a certain amount is short-chained or even in the monomeric state (G-actin). This actin equilibrium is rapidly and effectively controlled by the cell and obviously involved in such important phenomena as viscosity changes, cytoskeletal functions and motive force generation for motility. This actin equilibrium and its changes most likely influence many other cell physiological processes.

The occurrence of actin transformations within the contraction–relaxation cycle, i.e., actin depolymerisation at the end of the contraction process, complicates the analysis of the molecular contraction mechanism of cytoplasmic actomyosin. For a better understanding, a more thorough knowledge concerning its control must be gained. For regulation, different possibilities (in different cells) are discussed at present: troponin/tropomyosin, calmodulin, phosphorylation phenomena and so-called "actin-modulating proteins". Taken all together, the control of contraction phenomena also seems to be more complex than in cross-striated muscle.

Transformation of Motive Force into Locomotion

The primary motive force generated by a contraction mechanism of the cytoplasmic actomyosin results in a shortening of linear structures (actomyosin fibrils) or in a volume contraction of a network structure ("superprecipitation"). However, the locomotion of living cells must also be explained, i.e., a cytoplasmic mass transport over relative large distances. The primary motive force must be transformed into rather complex, regular and continuous movement phenomena. The similarities of cytoplasmic actomyosin and muscle actomyosin suggest similar contraction mechanisms, perhaps a sliding principle. Following this assumption, one could speculate that there would be a certain c o n f o r m a n c e in all eukaryotic cells concerning the primary motive force generation at the molecular level, when based on actomyosin. In contrast to this, it is obvious that the transformations of the primary generated forces into cytoplasmic mass transport and thereby locomotion are extremely d i v e r g e n t. This becomes apparent when comparing

the movement of different types of amebae and when comparing the locomotion of these free living protozoans with the migration of tissue culture cells. The best known phenomena, such as pressure flow of endoplasm, traction forces or shearing forces (*Nitella* and plant cells), show an extreme divergence concerning the transformation mechanism in spite of the fact that all may be based on cytoplasmic actomyosin. Thus, we have to deal separately with the molecular basis of motility, i.e., the primary chemo-mechanical energy transformation, and a supramolecular level (the transformation of the generated forces into locomotion or motility phenomena). These motility phenomena are much more complex than a linear contraction of actomyosin at the molecular basis.

For both, the molecular and the supramolecular level, *Amoeba proteus* represents a classical object of research. A. Grębecki's lecture will give us the present state of knowledge concerning the question "how contraction is transformed to locomotion". P. Satir will cover "tubulin-based motility in protozoa" and will present new results indicating the capacity of cytoplasmic microtubules to perform primary motive force generation. M. Sleigh will deal with the supramolecular aspects of tubulin-based motility, i.e., with the phenomena of transformation of primary motive force into the complex movement of cilia. In spite of the admirable efforts of the organizers of this Congress, it was not possible in the present situation to cover two further aspects of motility, namely the "Biochemical background of cytoplasmic actomyosin" and the function of "Ca^{++}-binding proteins in ciliates".

(References available upon request).

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Symposium E

Supramolecular Aspects of Amoeboid Movement

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We learned very much during the past decade about the nature and function of contractile proteins and the nature and cortical distribution of the motile apparatus in amoeba. The undeniable progress in these fields was, however, not sufficient to improve our deficient understanding of how the physico-chemical phenomenon of contraction produces the well known biomechanical events: the endoplasm streaming, the extension of new pseudopodia, and the retraction of posterior cell regions. It seems that a full understanding of movement is impossible without continuously completing the biochemical and ultrastructural data by physiological experimentation on living cells.

The classical concept of Mast (1926) that the endoplasm streaming follows the hydrostatic pressure gradients created by contraction of the ectoplasmic cylinder has been given much more restrictive meaning in the tail contraction theory of Goldacre and Lorch (1950). The limitation of contraction site to the caudal region was supposed to create the pressure difference necessary to promote the flow. This view is recently represented by Wehland et al. (1979) who maintain that cortex along the lateral body walls is non-contractile (Fig. 1 A).

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Although the cortical distribution of the contractile apparatus along the whole cell periphery is now a well established fact, the frontal zone contraction theory (Allen 1961, 1973) is also still advocated (Fig. 1 B). It denies the active contraction of cortical tube, but claims instead that the contraction of viscoelastic loop of frontal endoplasm pulls forward everything behind it. The hydrostatic pressure is refused any role in the transmission of the motive force. But this theory has certainly one big advantage: it localizes the control and steering of locomotion in the anterior cell region "where the behavioural events occur" (Allen 1968).

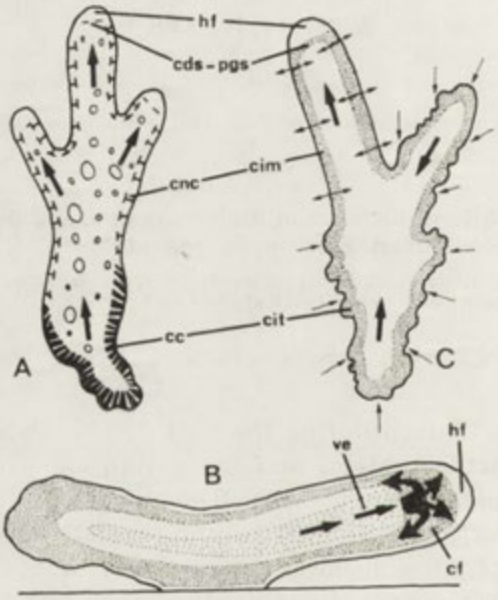

Fig. 1. Schemes explaining the three theories of amoeboid movement: the tail contraction (A — after Wehland et al. 1979), the frontal contraction (B — after Allen and Taylor 1975, simplified) and the generalized cortical contraction (after Grębecki, in press). Lettering: hf — frontal hyaloplasm, cds — "destabilized" cortex, pgs — plasmagel sheet, cnc — non-contractile cortex, cim — cortex contracting isometrically, cc — contractile cortex, cit — cortex contracting isotonically, cf — frontal contraction zone, ve — viscoelastic endoplasm

It seems that a correct theory of amoeboid movement should reconcile two elements: the motive force generation along the cell periphery where the contractile cortex is discovered, and the control of movement at the front of locomotion. It means that the motor and steering functions are probably separated, distributed between the cortical cylinder and the frontal zone. That is the basic idea of the generalized cortical contraction theory (Fig. 1 C) which has been proposed by us (Grębecki 1979, 1981, in press) and may be briefly exposed in the following lines.

The whole cortical cylinder, such as it is revealed by the electron microscopy, is contractile and contracting during locomotion. Its contraction is isotonic and performs work in the posterior regions, which are not firmly attached to the substratum and from which the endoplasm is easily evacuated. The adhesion and dilatation by the endoplasm inflow reduces the contraction of anterior regions to its isometric expression, when only the force is exerted. That force stabilizes the shape which permits a rise of intracellular pressure. If the contractile cylinder is under high internal pressure, a pressure drop at any point is sufficient to provoke the flow and to form a front of locomotion. The advancing fronts are then the only areas which are relaxed, and are under low pressure conditions. The relaxation of fronts or recovery of their contractility are under control of external stimuli. The resulting pressure changes in the frontal zone influence the whole pattern of streamings according to the hydrodynamic laws of flow. In that manner the fronts control the speed and the direction of movement, although the proper motive power is provided by all other parts of cell periphery behind them.

There are seven questions which seem to be crucial to test the validity of this concept against the tail and frontal contraction hypotheses.

(1) Is the Posterior Part of Cortical Tube Isotonically Contracting or Passively "Shortening"?

The changing size of different body parts of locomoting cell has been planimetrically evaluated by frame-by-frame film analysis (Grębecka and Grębecki 1975). In general, all the dimensions are decreasing in the posterior 2/3 of amoeba, whereas the anterior 1/3 is expanding. The decrease in size was interpreted as a manifestation of the local contraction under nearly isotonic conditions (Grębecki 1979). Allen and Allen (1978) sustain, however, that the decrease and withdrawal of the posterior regions is nothing else than a "shortening" due to the pulling force developed by the front.

The best way of testing both these interpretations is to check to what extent the motility of other regions behind the front is affected, when the frontal zone is suppressed by localized negative stimuli, or destroyed (Grębecki 1980, Grębecka 1981, Grębecka and Grębecki 1981).

Amoebae were allowed to move freely across a rectangular zone of shade (2500 lux). The shading filter was adjusted to such a position that they approached the illuminated zone (10 000 lux) in a corner of the

dark rectangle. Each new pseudopodium is then arrested after crossing the limits of the bright area. But the posterior regions continue to retract in the former direction, until all the possibilities of the endoplasm outflow are successively exhausted. It leads to dilatation of the anterior body part, which eventually tightly fills the angle of the shaded sector and remains imprisoned there for several minutes. Such a course of events strongly suggests that it is not the pulling activity of the front, but the persevered contraction of other body parts which squeezes the cell into the blind corner.

It might be, perhaps, objected that the frontal zones were not completely blocked. This ambiguity is avoided when the front is completely destroyed.

The whole frontal zone is destroyed after a brief contact with a micropipette filled with NaOH. The wound is seldom healed, and usually in its place an open breach is left in the peripheral cell layer. But the rear and middle body regions, which are intact, continue to contract and squeeze the endoplasm out, through the breach. The new endoplasm is still produced inside the tail, and gradually evacuated from the cell up to the total exhaustion of material and the full disintegration of amoeba. This "suicidal" behaviour unequivocally discards the hypothesis that the motility of amoeba depends on the pulling activity of its frontal zone, since the remaining body parts are capable of withdrawing, decreasing in size and producing the endoplasm streaming in the total absence of any front.

(2) Is the Contractility Limited to the Caudal Extremity?

The earlier morphometric study (Grębecka and Grębecki 1975) demonstrated that not only the uroid and the distal ends of old pseudopodia withdraw, but that the whole posterior and middle body part diminishes in size and is retracted. This visually perceptible isotonic contraction takes place behind the most stable adhesion sites and covers an area distinctly larger than postulated by the tail contraction theory.

The possibility that more anterior parts of cortex are also contractile (and contracting under isometric conditions) needed experimental testing. The localized photic stimulation (Mast 1932, Grębecki 1980) proved to be the appropriate technique to check the contracting effects of light and the relaxing effects of shade, when applied to various segments of the cortical cylinder (Grębecki 1981).

The cells adapted to shade were exposed to light limited to the uroid alone, to the posterior part of the cortical tube, to its central area, or to its anterior part except the proper front. In parallel, other cells were adapted to light and then exposed to local shading of the same four

segments. The effects were measured in the form of increased or decreased velocities of uroidal retraction and frontal extension.

The locomotion proved to be accelerated by illumination of any segment behind the front. The uroidal retraction is more influenced by stimulation of the posterior regions, whereas the frontal progression is most sensitive to contracting stimuli localized at the anterior end just behind the proper frontal zone. In general, however, the contractility is not limited to the tail but extended along the whole length of the cortical cylinder. These effects are additive. When the whole cortex, except the front, is exposed to the contracting influence of light, or relaxed by shade, the effects become more pronounced than in amoebae with the uroid stimulated alone. Whereas the acceleration by light proves that the whole cortex is contractile, its slackening in shade demonstrates on the other hand that the shaded areas were really contracting before the experiment, since they could be subsequently relaxed.

This last conclusion applies even to the "youngest" anterior part of the cylinder, which is supposed to be in the state of isometric contraction which stabilizes the cell shape and maintains the high intracellular pressure. Reinforcement of its contraction by light raises the frontal velocity to 152%, without much affecting the uroidal retraction. Its relaxation by shade, on the contrary, facilitates the endoplasm outflow from the tail, and therefore it raises to 129% the rate of uroidal retraction.

The isometric contraction of the anterior segment of cortical tube fits well with the pattern of microfilaments in that region. They are stretched and parallelized (Grębecka and Hrebenda 1979, Wehland et al. 1979), as in the plasmodial veins of *Physarum* fixed in the state of isometric contraction (Wohlfarth-Bottermann and Fleischer 1976) or in the stress fibers of fibroblasts (Goldman et al. 1976).

The contractility of the anterior cortex explains as well the bending movements of frontal pseudopodia which are occasionally seen in the horizontal plane (Rinaldi 1963, Grębecki 1977), and in the vertical plane are a regular component of locomotion, necessary to establish the new attachment sites (Bell and Jeon 1963). Bending of frontal pseudopodia toward the substratum is an active movement independent of gravitation (Nowakowska and Grębecki 1978).

Another demonstration of contractility of the whole cortex is brought by the glycerinated models of amoebae, which contract uniformly along their whole length (Rinaldi et al. 1975).

If the youngest layers of cortex are ready to contract, the actin repolymerization should probably begin earlier, already in the streaming endoplasm when it approaches the frontal zone. It my explain why this

portion of the endoplasm is viscoelastic and birefringent (Allen and Roslansky 1958 and 1959), and why actin fails to penetrate through the plasmagel sheet (Gawlitta et al. 1980).

(3) Are the Motor Phenomena Dependent on the Intracellular Pressure?

The contraction of the whole cortical cylinder, isotonic in the rear and isometric close to the front, may really create and maintain a high intracellular pressure, and thus accumulate the potential energy to be used in locomotion. Turgidity of amoeba was established already by the classical authors (Edwards 1923, Mast 1926), and their conclusion that the endoplasm streaming follows the pressure gradients is almost generally accepted from nearly 60 years.

The classical experiment of Edwards (1923) who perforated the body wall of amoeba, was recently repeated by Grębecka (1981). When a breach is produced at any place in the peripheral cylinder, the outflow of endoplasm has always at the beginning the eruptive character. If the tail region is perforated, the direction of streaming is gradually reversed and all the frontal pseudopodia are subsequently retreated. It means that the atmospheric pressure at the place of the injury, is lower than the hydrostatic pressure in the cell interior, and that the arising gradient is sufficient to promote the flow.

A low negative pressure of 2–5 cm of H_2O applied to the interior of the tail through a thin micropipette maintained inside the endoplasm also induces the streaming reversal (Grębecka 1980). The same pressure values are sufficient to control the streaming in amoebae kept in a double chamber (Kamiya 1964).

However, the frontal zone contraction theory rejects any role of pressure in generating the streaming. That view is supported by the experiment of Allen et al. (1971) who sucked one pseudopodium into a large micropipette and applied to its external surface a high negative pressure (35 cm of H_2O). The amoeba was still capable of developing pseudopodia in the opposite direction. Other authors expressed justified doubts whether the pressure applied externally is really transmitted to the cell interior. It was recently demonstrated by Grębecka (1980) that the suction force acting on the pseudopodium inside the micropipette stimulates its contraction, which transforms it into the tail of imprisoned amoeba. Therefore, under such conditions, amoeba cannot be considered as an inert hydrodynamic system, because it develops active resistance resulting in an escape reaction. So, this experiment cannot disprove the decisive role of pressure gradients in promoting and guiding the movement.

If the pressure inside the cell is higher than outside, its drop at any point around the periphery should provoke the streaming and initiate the formation of a new front.

(4) Are Lower Pressure Conditions Expectable in the Frontal Zone?

Lower pressure conditions must obviously arise in any place where the continuity of the contractile cortical envelope is disrupted. Then, the endoplasm streaming should be automatically directed toward the breach and form a new front.

Such a phenomenon has been first discovered in the monotactic cells. This particular type of "monopodial" amoeba has a huge hemispherical frontal cap, which content is watery (Korohoda and Stockem 1976) and which arises by fusion of cytoplasmic vacuoles (Grębecka 1978 a). It is in fact a vesicle enveloped with the membrane which could be demonstrated by micrurgical procedures (Grębecka 1978 b) and by electron microscopy (Hrebenda and Grębecka 1978). There is no trace of cortex around such a cap, which forms therefore a true breach in the contractile layer. During the spontaneous development of monotactic forms from the polytactic ones, the decisive transformation phase takes no more than a few seconds. When the vesicle is formed and pressed against the cell membrane, all the streamings are immediately reversed in that direction, and all earlier pseudopodia are retracted. A monotactic amoeba may recover the polytactic shape only after the ectoplasm is reappearing between the vesicular cap and the frontal membrane (Grębecka 1978 a, b). The flow toward the breach formed by the vesicular cap is so powerfull that monotactic amoebae are insensitive to external stimuli (Grębecki and Grębecka 1978).

This natural phenomenon may be simulated by injecting paraffin oil drops, according to the technique of Goldacre (1961), against the inner face of the cell membrane. The injected droplet immediately forms an artificial oil cap which also attracts all the streamings and transforms amoeba into an artificial monotactic form. Competition between two or more artificial and natural frontal caps produced in the same cell was studied (Grębecka 1977, 1978 b). The electron microscopy demonstrated that there is nothing more than the naked cell membrane with glycocalix between the oil and the outer medium (Hrebenda and Grębecka 1978). So, a breach in the cortical envelope proved again to be capable of reorganizing the streaming pattern and forming the front of locomotion.

The artificial fronts were also produced by local injections of the DNAase I which depolymerizes the F-actin (Wehland et al. 1979) and

of an actin-modulating protein from *Physarum* which inhibits its repolymerization (Gawlitta et al. 1980).

All these results have a common significance. Any local rupture or disintegration of the cortical tube creates a lower pressure spot which attracts the streaming and becomes the new front.

The frontal caps of normal polytactic amoebae are built of the hyaloplasm (Korohoda and Stockem 1975, 1976) which is separated from the granuloplasm by a semipermanent structure called the plasmagel sheet (Mast 1926, Rinaldi 1964 a, b). There is no organized cortex beneath the proper frontal membrane, but the filamentous threads are found deeper, between the hyaloplasm and the granuloplasm (Grębecka and Hrebenda 1979, Wehland et al. 1979). They may be certainly identified to the plasmagel sheet of light microscopists. The fluorescent actin, probably partly polymerized after injection, is found in abundance along the hyaloplasm-granuloplasm border but it fails to penetrate on the frontal cap territory (Gawlitta et al. 1980). The cortex detached from the membrane acts as a sieve through which the hyaloplasm may pass freely, as demonstrated by the phalloidin experiments of Stockem et al. (1978). If so, the frontal detached cortex which forms the plasmagel sheet offers only little resistance to the intracellular pressure created by those regions where it is anchored to the membrane. Therefore, a drop of pressure is in fact predictible in each advancing front, as postulated by the generalized cortical contraction theory.

(5) Is the Frontal Zone in the Relaxing or in the Contracting State?

The structure of the frontal zone allows us to suppose that the anterior end of a moving amoeba is in the relaxed condition. It is supported by investigations of living amoebae locally stimulated by contracting and relaxing agents. First indication was provided by local application of benzene, which relaxing influence is known not only in amoeba, and which provokes the development of a new front when is locally applied to the cell surface (Korohoda 1977).

More thoroughly was studied in this respect the contracting action of light and the relaxing influence of shade (Grębecki 1981). Their effects on four segments of the cortical cylinder, which were quoted above, were now compared to the results of their application to the front alone. The response elicited by the stimulation of front is always functionally opposed to the reaction produced by the same stimulus acting on any part of the cortical tube. Illumination of the front slows down its expansion, whereas the illumination of cortex accelerates it. And *vice versa*, a shaded front moves faster, but shading of cortex suppres-

ses its progression. It should be generalized that a contracting stimulus inhibits the locomotion when acting on the front, but activates it when acting anywhere else. A relaxing factor, on the contrary, favors the movement when applied to the front, and disfavors when applied to the cortex.

When the local action of light or shade is more extended in time, it produces spectacular behavioural effects. The light may stop any advancing pseudopodium as already reported by Mast (1932). The shade may initiate a new front in the lateral body wall, reactivate any contracting pseudopodium, assure to any advancing front the leading position and transform the polytactic amoeba into a "monopodial" orthotactic form. The alternating application of light and shade to any pseudopodial tip induces many subsequent reversals and re-reversals of streaming (Grębecki 1980).

The explanation of these phenomena on the basis of the frontal contraction theory needs the assumption that contraction is inhibited by light, whereas the shade activates or at least protects it. But that would be opposed to all the knowledge on the action of light on amoebae accumulated from over 50 years.

The effects of light and shade are, on the contrary, predictible by the generalized cortical contraction theory, because it postulates that the front only is in the state of relaxation, which may be reinforced or reversed by external stimuli.

(6) Are the Movements of Different Body Parts Mutually Related?

Two clearly opposed brief answers are given to this question. Amoeba "acts as an organized unit" said Mast (1932). Each pseudopodium "behaves as an independent functional unit" assert Allen and Allen (1978). Owing to that simple statement the frontal contraction theory lost its greatest merit, that of attributing the control functions to the front of amoeba. The front could not control the locomotion of the whole cell if its activity and the behaviour of other cell parts were unrelated.

There are many examples of functional interdependence of pseudopodia. For example, the formation of a huge pseudopodium headed by a natural vesicular cap or an artificial oil cap leads to the retreat of all other fronts (Grębecka 1977, 1978 a). The same result is obtained after relaxation of one pseudopodial tip by shade (Grębecki 1980, Grębecki and Kłopocka 1981).

The interdependence of pseudopodia was first studied cinematographically in freely moving unstimulated cells (Kłopocka and Grębecki 1980). The formation of each new front proved to be distinctly

correlated in time with the cessation of movement or the beginning of its reversal in some older pseudopodia. Velocities of sister pseudopodia fluctuate in the antagonistic way. These events cannot be under the control of the tail region, because in unstimulated amoebae it is retracted with a steady rate. They clearly depend on the interaction of different fronts.

The localized photic stimulation allowed us (Grębecki and Kłopocka 1981) to reveal what is here the primary event. Is a new front developed because an old one begun to retreat, or on the contrary, the formation of a new front initiates the withdrawal of some others? When a new front is induced by shade, the others retract after some delay. When an advancing front is inhibited by light, it slows down and stops but the retraction begins only after the formation of its successor. We confirmed also the observation of Allen (1973) that the streaming reversal begins at the new front and then it is gradually propagated up to the older one. It should be concluded that the formation of new fronts is the primary event which precedes the streaming reversal and the withdrawal of older pseudopodia.

These results contradict the pretended independence of pseudopodia, but they fit very well with the view that the front of amoeba is responsible for controlling and steering locomotion.

(7) Is the Front Responsible for Controlling and Steering Locomotion?

There are no data allowing us to presume reasonably any kind of transmission of impulses along the cell membrane of amoeba. The mechanism by which the distant body parts may react to stimuli acting elsewhere must be much simpler. As a matter of fact, if the endoplasm streamings follow the branched system of pressure gradients inside the cell, the elementary principles of hydrodynamics are sufficient to create a simple but efficient coordination of movement. Any change of flow conditions in one branch must necessarily affect the transport in other interconnected channels. The particularity of the present theory is the control of flow located at the low pressure poles of hydrostatic gradients, that is in the tips of advancing pseudopodia.

It explains why in the light-shade experiments (Grębecki 1981) the stimulation of the front usually affects the velocity of uroidal retraction as well as its own extension. When the leading pseudopodium is inhibited by light it obstructs the evacuation of endoplasm from the tail region, provided that new fronts were not yet formed. When the leading pseudopodium is activated by shade, or when their number is increased, the endoplasm outflow becomes easier and the uroid is faster

withdrawn. But according to the same principle, the increased number of fronts reduces the individual rate of expansion of each of them. These relationships were demonstrated in standardized bipodial amoebae (Kłopocka and Grębecki, in press). In that manner the fronts control the speed of locomotion.

Of course, they control as well the direction. The most common mode of guiding the movement is based on the correlation between pseudopodia, which was described above. When a positive stimulus activates one front, other directions are abandoned after a brief delay. When a negative stimulus stops it, it retreats after a new one is formed in another direction.

It was recently demonstrated (Grębecki et al. 1981) that it is possible to steer the whole cell locomotion by the stimuli applied asymmetrically to one single front, without affecting any other body part. When a growing tip of a pseudopodium is followed by a micropipette filled with benzene it eventually turns toward its orifice. When frontal pseudopodia of an amoeba migrating in shade penetrate unilaterally on a bright spot, they deviate out of it. An orthotactic amoeba may be guided along a bending path by continuously shading one side of its frontal zone. If it is obliged to follow a dark stripe forming a loop, the frontal zone regularly refines the direction of its further expansion and modifies its configuration according to the curvature of the loop. As a result, the shape of the whole cell becomes quite correctly adapted to the changing geometry of the preprogrammed path of locomotion.

In general, the experiments strongly support the postulation that the control and steering of movement in amoeba is the function of its frontal zone, which is repelled by contractants and attracted by relaxing factors.

It should be summarized that the frontal zone contraction theory is untenable, because it takes no account of the peripheral location of the contractile apparatus along the whole cell length, except of the frontal extremities; because it neglects the role of pressure gradients in generating and controlling the streamings; because instead of explaining the coordination of movements of different cell parts it denies its existence; because the front responds negatively to contracting stimuli and is attracted by the relaxing ones; and finally, because the inhibition and even the destruction of all fronts fails to arrest the motile activity of other regions.

The tail contraction theory seems not to be essentially wrong, but largely insufficient. It overlooks the motor events which prove the contractility of middle-anterior parts of the cortical tube, up to the confines of the frontal zone; it proposes no mechanism to control and steer

the cell migration, and in particular, it could be hardly reconciled with the behavioural evidence that the front first must respond to new situations encountered during locomotion.

The theory of the generalized cortical contraction tries to fill these gaps by admitting that the contractility of the whole cortex contributes to the increase of hydrostatic pressure in the cell interior. The movement is provoked, regulated, directed and coordinated by local drops of pressure in the pseudopodial fronts, which are under the control of external stimuli. According to this scheme, many observations which appeared before to be contradictory and were used as arguments in the classical dispute, find a common explanation.

REFERENCES

Allen R. D. 1961: A new theory of amoeboid movement and endoplasmic streaming. Exp. Cell Res., (Suppl.), 8, 17–31.

Allen R. D. 1968: Differences of a fundamental nature among several types of amoeboid movement. Symp. Soc. Exp. Biol., 22, 151–168.

Allen R. D. 1973: Biophysical aspects of pseudopodium formation and retraction. In: The Biology of *Amoeba* (ed. K. W. Jeon), Academic Press, 201–247, New York and London 1973.

Allen R. D. and Allen N. S. 1978: Cytoplasmic streaming in amoeboid movement. Ann. Rev. Biophys. Bioeng., 7, 469–495.

Allen R. D., Francis D. and Zeh R. 1971: Direct test of the positive pressure gradient theory of pseudopod extension and retraction in amoebae. Science, 174, 1237–1240.

Allen R. D. and Roslansky J. D. 1958: An anterior-posterior gradient of refractive index in the amoeba and its significance in amoeboid movement. J. Biophys. Biochem. Cytol., 4, 517–524.

Allen R. D. and Roslansky J. D. 1959: The consistency of amoeba cytoplasm and its bearing on the mechanism of amoeboid movement. I. An analysis of endoplasmic velocity profiles of *Chaos chaos* (L.). J. Biophys. Biochem. Cytol., 6, 437–446.

Allen R. D. and Taylor D. L. 1975: The molecular basis of amoeboid movement. In: Molecules and Cell Movement (eds. S. Inoue and R. E. Stevens), Raven Press, 239–258. New York, 1975.

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

Edwards J. G. 1923: The effect of chemicals on locomotion in *Amoeba*. J. Exp. Zool., 38, 1–43.

Gawlitta W., Hinssen H. and Stockem W. 1980: The influence of an actin-modulating protein (AM-protein) from *Physarum polycephalum* on the cell motility of *Amoeba proteus*. Eur. J. Cell Biol., 23, 43–52.

Gawlitta W., Stockem W., Wehland J. and Weber K. 1980: Organization and spatial arrangement of fluorescein-labeled native actin, microinjected into normal locomoting and experimentally influenced *Amoeba proteus*. Cell Tiss. Res., 206, 181–191.

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, 487–499.

Goldman R. D., Schloss J. A. and Starger J. M. 1976: Organizational changes of actinlike microfilaments during animal cell movement. In: Cell

Motility (eds. Goldman R. D. et al.), Cold Spring Harbor Laboratory, 217–245, Cold Spring Harbor 1976.
Grębecka L. 1977: Changes of motory polarization in *Amoeba proteus* as induced by oil injections. Acta Protozool., 16, 107–120.
Grębecka L. 1978a: Frontal cap formation and origin of monotactic forms of *Amoeba proteus* under culture conditions. Acta Protozool., 17, 191–202.
Grębecka L. 1978b: Micrurgical experiments on the frontal cap of monotactic forms of *Amoeba proteus*. Acta Protozool., 17, 203–212.
Grębecka L. 1980: Reversal of motory polarity of *Amoeba proteus* by suction. Protoplasma, 102, 361–375.
Grębecka L. 1981: Motory effects of perforating peripheral cell layers of *Amoeba proteus*. Protoplasma, 106, 343–349.
Grębecka L. and Grębecki A. 1975: Morphometric study of moving *Amoeba proteus*. Acta Protozool., 14, 337–361.
Grębecka L. and Grębecki A. 1981: Testing motor functions of the frontal zone in the locomotion of *Amoeba proteus*. Cell Biol. Internat. Rep., 5, 587–594.
Grębecka L. and Hrebenda B. 1979: Topography of cortical layer in *Amoeba proteus* as related to the dynamic morphology of moving cell. Acta Protozool., 18, 493–502.
Grębecki A. 1977: Non-axial cell frame movements and the locomotion of *Amoeba proteus*. Acta Protozool., 16, 53–85.
Grębecki A. 1979: Organization of motory functions in amoebae and in slime moulds plasmodia. Acta Protozool., 18, 43–58.
Grębecki A. 1980: Behaviour of *Amoeba proteus* exposed to light-shade difference. Protistologica, 16, 103–113.
Grębecki A. 1981: Effects of localized photic stimulation on amoeboid movement and their theoretical implications. Eur. J. Cell Biol., 24, 163–175.
Grębecki A.: Études expérimentales sur la localisation des fonctions motrices chez les amibes. Année Biol., (in press).
Grębecki A. and Grębecka L. 1978: Morphodynamic types of *Amoeba proteus*: a terminological proposal. Protistologica, 14, 349–358.
Grębecki A., Grębecka L. and Kłopocka W. 1981: Testing steering functions of the frontal zone in the locomotion of *Amoeba proteus*. Cell Biol. Internat. Rep., 5, 595–600.
Grębecki A. and Kłopocka W. 1981: Functional interdependence of pseudopodia in *Amoeba proteus* stimulated by light-shade difference. J. Cell Sci., 50, 245–258.
Hrebenda B. and Grębecka L. 1978: Ultrastructure of the frontal cap of monotactic forms of *Amoeba proteus*. Cytobiologie, 17, 62–72.
Kamiya N. 1964: The motive force of endoplasmic streaming in the amoeba. In: Primitive Motile Systems in Cell Biology (eds. Allen R. D. and Kamiya N.), Academic Press, 257–277, New York and London 1964.
Kłopocka W. and Grębecki A. 1980: Motory interdependence of pseudopodia in freely moving *Amoeba proteus*. Acta Protozool., 19, 129–142.
Kłopocka W. and Grębecki A.: Locomotion of *Amoeba proteus* after standardizing its body shape. Protoplasma (in press).
Korohoda W. 1977: Experimental induction of locomotion in enucleated fragments of *Amoeba proteus* and its bearing on the theories of amoeboid movement. Cytobiologie, 14, 338–349.
Korohoda W. and Stockem W. 1975: On the nature of hyaline zones in the cytoplasm of *Amoeba proteus*. Microsc. Acta, 77, 129–141.
Korohoda W. and Stockem W. 1976: Two types of hyaline caps, constricting rings and the significance of contact for the locomotion of *Amoeba proteus*. Acta Protozool., 15, 179–185.
Mast S. O. 1926: Structure, movement, locomotion and stimulation in *Amoeba*. J. Morphol., 41, 347–425.
Mast S. O. 1932: Localized stimulation, transmission of impulses, and the nature of response in *Amoeba*. Physiol. Zool., 5, 1–15.
Nowakowska G. and Grębecki A. 1978: Attachment of *Amoeba proteus* to the substrate during upside-down crawling. Acta Protozool., 17, 353–358.
Rinaldi R. A. 1963: Velocity profile pictographs of amoeboid movement. Cytologia, 28, 417–427.

Rinaldi R. A. 1964 a: Pictographs and flow analysis of the hyaline cap in *Chaos chaos*. Protoplasma, 58, 603–620.
Rinaldi R. A. 1964 b: The plasmagel sheet of *Amoeba proteus*. Protoplasma, 59, 480–484.
Rinaldi R. A., Opas M. and Hrebenda B. 1975: Contractility of glycerinated *Amoeba proteus* and *Chaos chaos*. J. Protozool., 22, 286–292.
Stockem W., Weber K. and Wehland J. 1978: The influence of microinjected phalloidin on locomotion, protoplasmic streaming and cytoplasmic organization in *Amoeba proteus* and *Physarum polycephalum*. Cytobiologie, 18, 114–131.
Wehland J., Weber K., Gawlitta W. and Stockem W. 1979: Effects of the actin-binding protein DNAase I on cytoplasmic streaming and ultrastructure of *Amoeba proteus*. An attempt to explain amoeboid movement. Cell Tiss. Res., 199, 353–372.
Wohlfarth-Bottermann K. E. and Fleischer M. 1976: Cyclic aggregation patterns of cytoplasmic F-actin coordinated with oscillating tension force generation. Cell Tiss. Res., 165, 327–344.

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Symposium E

Tubulin-Based Motility in *Protozoa*

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Tubulin

Tublin is the general name for a class of ca. 50 kd proteins of eukaryotic cells. In organisms that have been looked at closely, there are several tubulin genes per cell and the slightly different tubulins may have slightly different properties. Tubulin usually occurs in the cytoplasmic and nucleoplasmic matrix as a heterodimer of 100 kdalton containing two subunits designated α and β with binding sites for guanine nucleotides. Diagnostic characteristics of the protein include a colchicine binding site and the ability to self assemble *in vitro* into microtubules under appropriate environmental conditions. Conditions that promote assembly include GTP, relatively elevated temperature (37°C) and low Ca^{2+} ($< 10^{-7}$M). At 0° or with elevated Ca^{2+}, microtubules disassemble into their tubulin subunits. This cycle of assembly-disassembly can be used to purify the protein, although certain microtubule-associated proteins (MAPs) will usually co-purify with tubulin.

Microtubules are composed of rows of tubulin subunits, called protofilaments. During assembly *in vitro*, a sheet of 13 protofilaments rolls

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up into the completed microtubule. After nucleation, the microtubule often grows from both ends depending on the concentration of free tubulin. However, the ends are not alike, in that growth is much faster at one than the other. When ciliary microtubules are used for nucleation in such experiments, the fast polymerizing end or plus (+) end corresponds to the tip and the slow polymerizing (−) end corresponds to the base of the cilium.

At steady state, microtubules remain at constant length in equilibrium with tubulin dimers. Under these conditions, there is net addition of tubulin at the fast polymerizing (+) end, probably accompanied by GTP hydrolysis, and net loss of tubulin at the slow (−) end. This is illustrated in Fig. 1. This condition, which has its analog in the actin

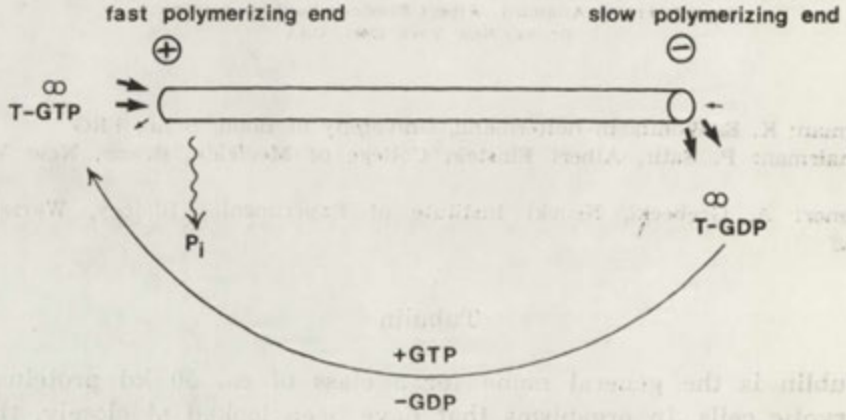

Fig. 1. Microtubule treadmilling. Each microtubule has a fast polymerizing (+) and slow polymerizing (−) end. At steady state, tubulin dimers (T-GTP) add to the (+) end; presumably hydrolysis of GTP accompanies addition. A net disassembly of T-GDP dimers occurs at the (—) end. With time, a given tubulin dimer moves along the microtubule from (+) to (−) end, as on a treadmill. T-GTP is regenerated in the free tubulin pool by nucleotide exchange. (Based in part on Margolis and Wilson 1978)

microfilament, causes a given tubulin molecule to flux through the microtubule from (+) to (−) end with time and has been called 'treadmilling' (Margolis and Wilson 1978). *In vivo*, both nucleation and growth of microtubules may be very stringently controlled (see article by Tucker, this volume).

Motility Based on Tubulin Assembly Properties

The assembly-disassembly properties of tubulin are important in developing and maintaining cell shape and can be used to produce organelle displacement and limited cell movement, since assembly often

is accompanied by etxension of cell processes and disassembly by their retraction or disappearance. One of many examples which have been studied in protozoan and metazoan cells is the heliozoan *Heterophrys marina* axopod (Davidson 1975), which contains six microtubules hexagonally arranged around a central fiber. In the presence of external Ca^{2+} and a mechanical stimulus, the axopod rapidly contracts, presumably because an influx of Ca^{2+} across the axopod membrane simultaneously induces microtubule depolymerization and contraction of the central fiber. The axopod then reextends slowly as microtubules reassemble, elongating to their resting length which can be as much as 70 μm, presumably after the Ca^{2+} is pumped from the cell cytoplasm. Haptocysts attached to the membrane move with the contracting and elongating axopod.

The mitotic spindle also appears by microtubule assembly and disappears when the microtubules disassemble. This has placed assembly-disassembly mechanisms in the center of several theories of spindle elongation and chromosome movement, and has focussed attention on the transient nature of microtubules. While this attention is not misplaced, the lifetime of certain microtubules, such as those of cilia or of the various cortical bundles in protozoa, is relatively long. These microtubules are considered stable despite the fact that they too can be rapidly disassembled under the appropriate conditions, as for example, the axonemal microtubules are disassembled during flagellar resorption in *Chlamydomonas*. What seems probable is that tubule assembly is only the first step in producing a biologically functional microtubule. Microtubules may be stabilized against disassembly by interactions of tubulin with other proteins, which may block the ends of the microtubule or perhaps lock the structure together at the defined length.

Microtubule Sliding in Cilia

Some forms of cell motility are produced by interactions of assembled stabilized microtubules with one another, most generally by microtubule sliding. The sliding microtubule mechanism as a device for the production of movement is best known for cilia, and protistan cilia have played a significant role in the experimental elucidation of the mechanism. The ciliary axoneme contains 9 doublet microtubules and a central single pair. Although compound microtubules such as the axonemal doublets are a special feature of cilia and related centriolar derivatives, doublet microtubules are not fundamentally different from other microtubules. The doublets contain a complete microtubule of 13 protofilaments (subfiber A) onto which an incomplete microtubule assembles (subfiber B). Heidemann and McIntosh (1980) have recently

succeeded in growing protofilament ribbons onto ordinary cytoplasmic microtubules. These are essentially incomplete B subfibers. Often multiple ribbons grow on one microtubule, but if a single initiation point were specified, closure would produce a doublet. Incidentally, the ribbons also can be used to define microtubule polarity; the large majority grow with a clockwise curvature when viewed from tip (+) to base (−) of the microtubule (see Fig. 2).

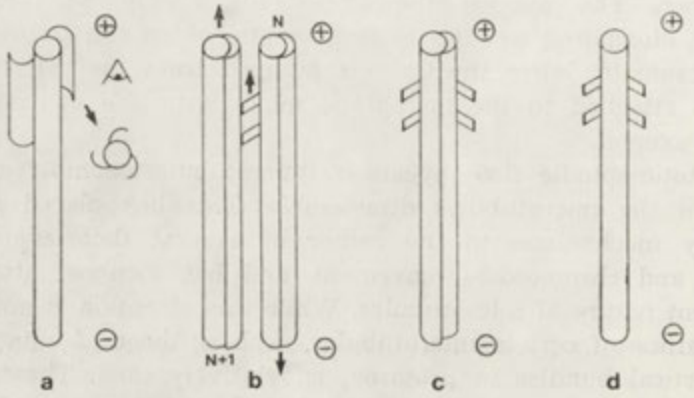

Fig. 2. Microtubule decoration and polarity. (a) Decoration by partial microtubules grown on cytoplasmic microtubules. The curvature of growth is predominantly in one direction, clockwise when viewed from the (+) end to the microtubule. (Based largely on Heidemann and McIntosh 1980). (b) *In situ* decoration of ciliary doublets by dynein. The dynein arms attach to subfiber A of each axonemal doublet (N). They tilt baseward and act to move doublet N + 1 in a (+) direction relative to doublet N. (Based on Sale and Satir 1977). (c) *In vitro* decoration of axonemal doublets by *Tetrahymena* dynein. Arms decorate both A and B subfibers: in both cases, they tilt baseward, producing an arrowhead in the tipward (+) direction. (Based on Takahashi and Tonomura 1978). (d) *In vitro* decoration of cytoplasmic microtubules by *Tetrahymena* dynein. Multiple rows of tilted arms bind; the tilt is apparently identical to that seen for axonemal doublets. (Based on Satir et al. 1981)

The sliding microtubule interaction between ciliary doublets has been studied in considerable detail (cf. Satir 1979). Sliding is powered by the action of the dynein arms extending from one doublet (N) to its neighbor (by convention, N + 1). The arms are constitutively assembled onto subfiber A of doublet N and interact with subfiber B of doublet N + 1. Sale and Satir (1977) studying sliding between *Tetrahymena* doublet microtubules showed that extended but unattached arms of doublet no. N tilt toward the base (−) end of the doublet and that in sliding, doublet N always moves relatively in the (−) direction, while N + 1 moves relatively in the (+) direction (see Fig. 2).

Takahashi and Tonomura (1978) have used isolated *Tetrahymena* dynein to decorate axonemal microtubules that have been stripped of dynein. They showed that dynein arms will bind to both subfiber A

and to subfiber B of each doublet. This decoration can also be used to identify microtubule polarity since both arms tilt in the same way (towards the base of the doublet) (see Fig. 2). Evidently, all axonemal microtubules have their (+) and (−) ends aligned, i.e., are parallel. This is an important conclusion, since sliding has sometimes been thought to require antiparallel microtubules, as in some models of actin-myosin interaction. The polarity of arm attachment is intimately related to the single polarity of active sliding in the axoneme mentioned above. Using this information, we have been able to construct and test a structurally explicit mechanochemical cycle of the dynein arm that conforms to the single polarity of active sliding (Satir et al. 1981). In this cycle, the tilted dynein arm attaches to subfiber B of doublet N + 1 and moves tipwards, in a (+) direction with a sliding step of 16 nm. Consequently, doublet N + 1 is pushed relatively tipward, while doublet N moves relatively baseward in reaction. Binding of a new ATP allows the end of the attached arm to come free again; upon hydrolysis of ATP, the arm retilts and the cycle is completed.

In applying the results from ciliary studies to other cases where microtubule sliding may occur, we would anticipate that two major requirements of any sliding microtubule system would be: (1) parallel polarity of microtubule organization and (2) the presence of dynein or dynein-like molecules capable of undergoing a cross-bridge cycle in the presence of ATP. Presumably, dynein would interact with the assembled microtubules. Where these requirements were met, we would predict the direction of movement for the sliding system, that is microtubules bearing active arms will be displaced toward their (−) end during sliding.

Cytoplasmic Microtubules Can Bind Dynein

Strong tests for sliding potential of a system of cytoplasmic microtubules are therefore available. It is now known that cytoplasmic microtubule arrays often have parallel polarity, and recent evidence is available that some cytoplasmic microtubules can bind dynein. For example, brain microtubules polymerized *in vitro* will decorate with *Tetrahymena* dynein (Satir et al. 1981). This decoration is somewhat discontinuous, occurring in stretches, although more than one row of arms can be bound to the same microtubule. Preliminary results with negative stain preparations indicate that the tilt of the arms corresponds to that on axonemal microtubules (Fig. 2). This in turn supports the possibility that a mechanomorphological cycle similar to that of axonemal microtubules may operate for cytoplasmic microtubules. Using thin section techniques, Telzer and Haimo (1981) report that *Te-*

trahymena dynein will also bind to microtubules of the mitotic spindle, and Haimo et al. (1979) have shown that *Chlamydomonas* dynein will also decorate cytoplasmic microtubules.

As discussed further in Satir (1982), it seems likely that microtubule sliding operates in some systems of tubulin-based cell motility other than cilia. Certain protozoan microtubular arrays serve as especially good candidates for such systems. What is important and novel in the approach outlined here is that from polarity considerations, we can predict (1) which microtubules in the array should bear active arms (2) which direction these arms should tilt and (3) in which direction relative movement should occur. These predictions are reviewed in Fig. 3.

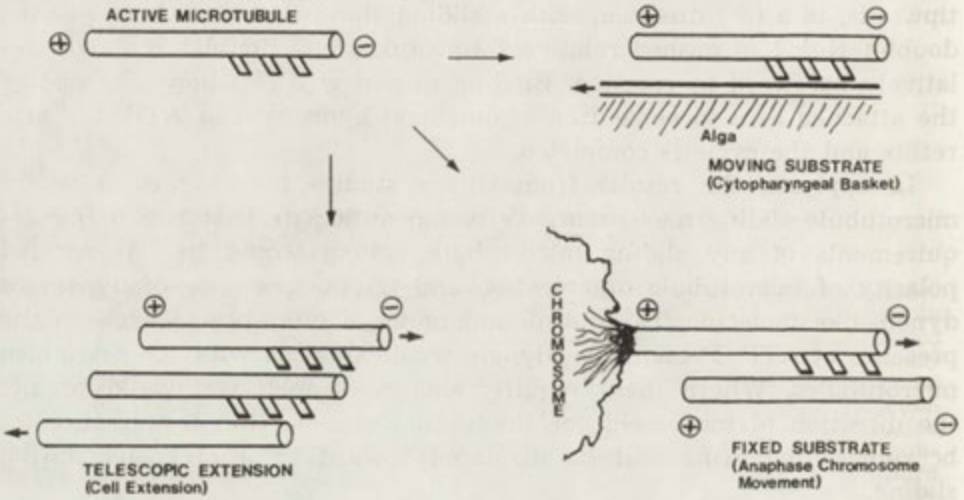

Fig. 3. Polarity predictions for active sliding of cytoplasmic microtubules of protozoan systems discussed in text. In each case, arrows indicate direction of predicted movements. (From Satir P. 1982, Courtesy Cold Spring Harbor Press)

Other Candidates for Sliding Microtubule-Based Motility

I mention only a few protozoan arrays here as illustrations:

(1) The axostyle of flagellates (e.g., *Saccinobaculus*) from the wood-eating roach or termite gut. The axostyle consists of ribbons of single microtubules linked together by two sets of bridges: interrow projections and intrarow bridges. It is not entirely certain which of these is dynein, but since adjacent ribbons are thought to slide during movement (Woodrum and Linck 1980) the best guess is that the interrow projections correspond to dynein, while the intrarow bridges are elastic links. The intact axostyle executes complex bending and propagates waves along its length. Axostylar microtubules have parallel polarity.

Mooseker and Tilney (1973) isolated axostyles from cells by Triton X-100 extraction. Addition of 2 mM ATP in appropriate solutions reactivated the isolated axonemes so that normal motility was restored. The specific conditions for reactivation were essentially identical to those that reactivate isolated ciliary axonemes. In SDS-PAGE, proteins of the isolated axostyle comigrate with authentic ciliary proteins; bands corresponding to tubulin and to dynein are evident. We may conclude (1) that the axostyle contains a molecular much like authentic dynein (2) that this dynein is capable of binding to single microtubules (3) that the axostyle meets the criteria for a sliding microtubule system, and in fact shows that microtubule sliding will operate to produce motion in arrays of single stable microtubules, as opposed to axonemal doublets. It will be interesting to test the predictions of relative sliding direction and arm action with this organelle.

(2) Km fibers in *Stentor*. The contraction-elongation cycle of *Stentor* has been reviewed by Huang and Mazia (1975). Telescopic microtubule sliding (see Fig. 3) operates to produce elongation, after a Ca^{2+} induced cell shortening. The microtubule ribbons (km fibers) of contracted cells show considerable overlap that is greatly reduced in extended cells. All microtubules probably have parallel polarity since each ribbon originates at a basal body. If, as elsewhere, the basal body is the (−) end of the microtubule, activity of an anterior ribbon would produce movement of the next ribbon toward the posterior of the organism, leading to elongation. There are morphological cross-bridges resembling dynein arms between adjacent ribbons. When the arm-bearing microtubules of successive ribbons are sectioned, the permanently attached end of the arm always is seen to one side of the microtubule, the detachable end to the other. The arms are tilted. It would be useful to correlate these directions and tilts with the anterior-posterior axis of the organism to test the predictions of active sliding. The biochemistry of the system is unknown.

(3) The micronuclear mitotic spindle in *Tetrahymena*. The intramicronuclear elongation and anaphase separation of kinetochores of the mitotic spindle in *Tetrahymena* has recently been carefully studied by LaFountain and Davidson (1980). During this event, kinetochore microtubules translocate from the equator of the spindle toward the spindle poles without any apparent change in the number or length. One possibility is that this occurs by a sliding microtubule mechanism. Cross-bridges between microtubules are sometimes seen. Pratt et al. (1980) have shown that sea urchin spindles contain high molecular weight polypeptides with dynein-like properties. Sakai et al. (1976) and Cande (1978) have prepared permeabilized cells in which anaphase chromosome movement is preserved. Antidynein and vanadate, a potent

dynein inhibitor, block such movement, but antimyosin does not. In the mitotic apparatus, assembly-disassembly and sliding of microtubules are probably both important to relative movements. Since disassembly and sliding are interdependent, dissection of the mechanism has proven difficult. The *Tetrahymena* micronuclear mitotic spindle has several advantages for such a dissection. In other organisms, it is now known that within a half spindle all microtubules have parallel polarity, with the (+) end at the metaphase kinetochore (Euteneuer and McIntosh 1981). This would predict that the kinetochore microtubules bear active dynein arms, and move poleward as shown in Fig. 3.

(4) The cytopharyngeal basket of certain ciliates. These structures have been studied in detail by Tucker (1978) and Hausmann and Peck (1979). The basket invaginates dramatically when the ciliate, for example *Pseudomicrothorax*, feeds. Filamentous algae are moved into the cell at rapid rates: membrane, algae and cytoplasm stream inward during this process. In the cytoplasm adjacent to the invaginating cell membrane lie single ribbons of microtubules (the nemadesmal lamellae) that bear rows of arms reminiscent of dynein. Using histochemical techniques, Hausmann (personal communication) has recently shown ATPase activity in the vicinity. If such arms were dynein-like, sliding would occur here between a fixed microtubule and the moving cell mebrane. Figure 3 predicts the arm and microtubule polarity in relation to the motion; these have not yet been experimentally determined. Tucker (personal communication) argues that especially in *Nassula* the distance between the moving membrane and the arms is too great for the proposed sliding system to operate. It may be that the arms act on peripheral proteins attached to the membrane rather than directly with membrane sites themselves. At the moment, while the situation is unresolved, the cytopharyngeal basket seems a potential model for cytoplasmic streaming, membrane flow or vesicular and mitochondrial movement based on a sliding microtubule mechanism. A similar system probably operates in the suctorian tentacle.

Conclusions

Tubulin-based motility is a ubiquitous feature among the protista. In many cases this is based primarily on the reversible assembly-disassembly of microtubules, which particularly influences cell shape and the production or elimination of (a) microtubule-based cell extensions such as axopodia or (b) internal arrays such as the mitotic apparatus. The stability of a given set of microtubules depends on a variety of cellular controls, including the local concentration of tubulin and numerous

microtubule-associated proteins that influence location, length, polarity, treadmilling, clustering or cross-linking of the assembling microtubule. Relatively stable sets of microtubules are found in protozoan cells. At least one type of widespread tubulin-based motility, ciliary motility, depends upon such a stable array and the interaction of the doublet axonemal microtubules with a mechanochemical transducer molecule, dynein, to produce relative sliding. The objection that the doublet axonemal microtubules are special seems muted by the demonstration that dynein will bind to cytoplasmic microtubules, and by the axostyle, an array of cytoplasmic microtubule-ribbons which bear arms resembling dynein and whose biochemistry suggests that the system operates in nearly identical fashion to the ciliary axoneme. Stringent criteria are now available to test for tubulin-dynein based microtubule sliding and these may be used in the next few years to determine in a definitive way whether such sliding occurs for a number of systems. The criteria include (1) parallel polarity of the microtubules in the putative sliding system (2) the presence of dynein or dynein-like molecules demonstrated by both structural and biochemical methods and (3) displacement of active microtubules in a (−) direction. The protozoa provide a number of especially attractive candidates for sliding microtubule arrays which embody general principles and which remain to be tested further.

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REFERENCES

Cande W. Z. 1978: Chromosome Movement in Lysed Cells in Cell Reproduction. (eds. Dirksen E. R., Prescott D. M. and Fox C. F.) Acad. Press. N. Y., pp. 457–464.

Euteneuer U. and McIntosh J. R. 1981: Structural polarity of kinetochore microtubules in PtK_1 cells. J. Cell Biol., 89, 338–345.

Davidson L. S. 1975: Studies of the actinopods *Heterophrys marina* and *Ciliophrys marina*: energetic and structural analysis of their contractile axopodia, general ultrastructure and phylogenetic relationships. Ph. D. Thesis, Univ. of California, Berkeley, 186 pp.

Haimo L. T., Telzer B. R. and Rosenbaum J. L. 1979: Dynein binds to and crossbridges cytoplasmic microtubules. Proc. Natl. Acad. Sci., (USA) 76, 5759–5763.

Hausman K. and Peck R. K. 1979: The mode of function of the cytopharyngeal basket of the ciliate *Pseudomicrothorax dubius*. Differentiation, 14, 147–158.

Heidemann S. R. and McIntosh J. R. 1980: Visualization of the structural polarity of microtubules. Nature, (Lond.) 286, 517–519.

Huang B. and Mazia D. 1975: Microtubules and filaments in ciliate contractility

in Molecules and Cell Movements. (eds. Inoue S. and Stephens R. E.) Raven Press, N. Y., pp. 389–409.
LaFountain J. R. Jr. and Davidson L. A. 1980: An analysis of spindle ultrastructure during anaphase of micronuclear division in *Tetrahymena*. Cell Motility, 1, 41–61.
Margolis R. L. and Wilson L. 1978: Opposite and assembly and disassembly of microtubules at steady state *in vitro*. Cell, 13, 1–8.
Mooseker M. S. and Tilney L. G. 1973: Isolation and reactivation of the axostyle. Evidence for a dynein-like ATPase in the axostyle. J. Cell Biol., 56, 13–26.
Pratt M. M., Otter T. and Salmon E. D. 1980: Dynein-like Mg^{2+}-ATPase in mitotic spindles isolated from sea urchin embryos (*Strongylocentrotus droebachiensis*). J. Cell Biol., 86, 738–745.
Sakai H., Mabuchi I., Shimoda S., Kuriyama R., Ogawa K. and Mohri H. 1976: Induction of chromosome motion in the glycerol-isolated mitotic apparatus: nucleotide specificity and effects of anti-dynein and myosin sera on the motion. Develop. Growth Differ., 18, 211–219.
Sale W. S. and Satir P. 1977: Direction of active sliding of microtubules in *Tetrahymena* cilia. Proc. Natl. Acad. Sci. (USA), 74, 2045–2049.
Satir P. 1979: Basis of flagellar motility in spermatozoa: current status. In: The Spermatozoon. (eds. Fawcett D. W. and Bedford J. M.). Urban and Schwarzenberg, Baltimore-Munich, pp. 81–90.
Satir P. 1982: Approaches to potential sliding mechanisms of cytoplasmic microtubules. In: The Organization of the Cytoplasm, Cold Spring Harbor Symp., Quant. Biol., 46, 285–291.
Satir P., Wais-Steider J., Lebduska S., Nasr A. and Avolio J. 1981: The mechanochemical cycle of the dynein arm. Cell Motility, 1, 303–327.
Takahashi M. and Tonomura Y. 1978: Binding of 30S dynein with the B-tubule of the outer doublet of axonemes from *Tetrahymena pyriformis* and the adenosine triphosphate-induced dissociation of the complex. J. Biochem. (Tokyo), 84, 1339–1355.
Telzer B. R. and Haimo L. T. 1981: Decoration of spindle microtubules with dynein: evidence of uniform polarity. J. Cell Biol., 89, 373–378.
Tucker J. B. 1978: Endocytosis and streaming of highly gelated cytoplasm alongside rows of arm-bearing microtubules in the ciliate *Nassula*. J. Cell Sci., 29, 213–232.
Woodrum D. T. and Linck R. W. 1980: Structural basis of motility in the microtubule axostyle: implications for cytoplasmic microtubule structure and function. J. Cell Biol., 87, 404–414.

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Symposium E

The Use of Cilia by *Protozoa*

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The primary function of cilia is the propulsion of fluids. The majority of protozoa live in water, and those that possess cilia or flagella use them to propel water. The water flow thereby created may cause the locomotion of the protozoan or provide a means of food collection or serve for both locomotion and feeding. The collection of food particles from water currents requires a mechanism for intercepting and capturing or filtering out the particles. Flagellates use a variety of ancillary structures for separating food particles from the water current, and some ciliates use special structures like extrusomes for food capture, but in general they use a filter made of cilia to separate out suitable particles. The use of flagella in water propulsion and food collection will not be discussed here because it has recently been reviewed elsewhere (S l e i g h 1981). This contribution will briefly describe how cilia propel water, how this propulsive activity is used in swimming and the creation of feeding currents and how some ciliary filter systems are believed to function.

The Propulsion of Water by Cilia

Cilia perform a beat cycle consisting of an extended effective stroke and a curved recovery stroke (Fig. 1), in a simple case these could be

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regarded as taking place in the same plane, perpendicular to the cell surface. As the cilium moves, each segment of it carries with it a surrounding zone of water whose extent decreases progressively the closer the moving segment of the cilium is to the cell surface (Blake 1972, Blake and Sleigh 1974). In the effective stroke therefore the extended cilium carries a much larger volume of water (from left to right in Fig. 1) than is carried back by the recovery stroke (from right to left in Fig. 1), because in the latter the cilium moves closer to the cell sur-

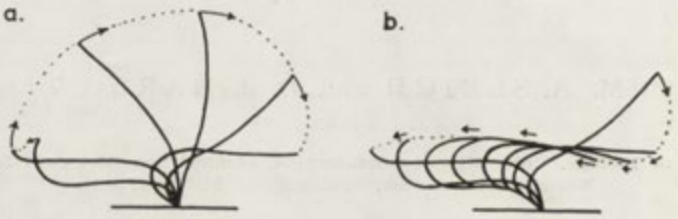


Fig. 1. Effective (a) and recovery (b) strokes of a ciliary beat cycle. The dotted line shows the path traced by the ciliary tip

face. There is therefore a net transport of water in the direction of the effective stroke in each cycle of beat (see also Sleigh 1976).

The difference between the volume of water carried forward by effective and backward by recovery strokes is maximised in ciliate pro-

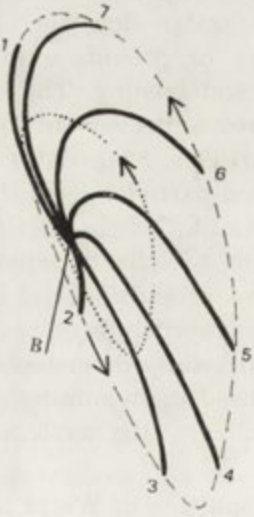


Fig. 2. View from above of the shape and position of the cilium of a ciliate at stages through the beat cycle. In the recovery stroke (from stages 3 through 6 to 1) the cilium follows a counter-clockwise sweep close to the cell surface, but in the effective stroke (from stages 1 to 3) the cilium is more erect. The ciliary base is at B

tozoa because in the recovery stroke the cilium moves to one side of the plane of the effective stroke and swings round close to the cell surface, with the result that a minimal volume of water is transported by the recovery stroke. In ciliates the recovering cilium always swings to the left of the direction of the effective stroke, so that the ciliary tip traces a counterclockwise path when viewed from above the cell surface (Fig. 2). The volume of water transported can also be increased by increasing the length of the cilium, but as ciliary length increases efficiency of propulsion decreases because the longer cilium bends backwards as it meets greater resistance from the water. Increased ciliary stiffness can be provided by grouping several or many cilia together to form a compound structure, and such longer, stiffer, compound cilia provide the main current-producing organelles of the larger ciliates.

Mutual assistance between cilia in the propulsion of water can also be provided by the movement of closely spaced cilia in coordinated waves. This can be important both in the production of higher instantaneous water velocities and in the maintenance of a continuous water flow. Where several cilia stand close enough to each other for their areas of influence in the surrounding water to overlap, then more of the energy released by activity within the cilia is transferred to effective propulsion of water. This is because less work is done against viscous shear resistance by a group of such close-set cilia moving more or less in phase than by the same number of isolated cilia. It is also important that metachronally coordinated cilia moving in continuous waves tend to maintain a continuous flow of water, and thereby give more efficient water propulsion because each beating cilium adds more energy to water that is already moving, rather than having to accelerate water from rest in each cycle of beat; this gives higher speeds of flow and fewer (or at least different) viscous losses (Sleigh and Aiello 1972, Sleigh and Barlow 1980).

These hydrodynamic interactions between metachronally coordinated cilia are also believed to be responsible for the coordination that forms metachronal waves (Machemer 1974). Where the areas of water moved by two adjacent cilia overlap, then the movement of either cilium must influence the other. The extent of influence must depend on the spacing between the cilia and the speed of movement. Usually the cilia of a fairly closely spaced row arranged in the plane of the effective stroke will beat more or less in phase (i.e., in synchrony), especially if the beat is vigorous. Neighbouring cilia will also influence one another in their recovery strokes, especially if the cilium swings strongly to one side in this phase of the beat. In ciliates, because the recovery stroke moves away to the left of the direction of the effective stroke, each cilium tends

to exert some influence on the next cilium on this side; there is some delay in this interaction and the cilia tend to move in sequence (metachronally) in this direction. Ciliate protozoa thus show dexioplectic metachrony (if cilia beat directly towards an observer, the waves move towards his right, see Figs. 3–6).

Use of Cilia in Swimming by Ciliates

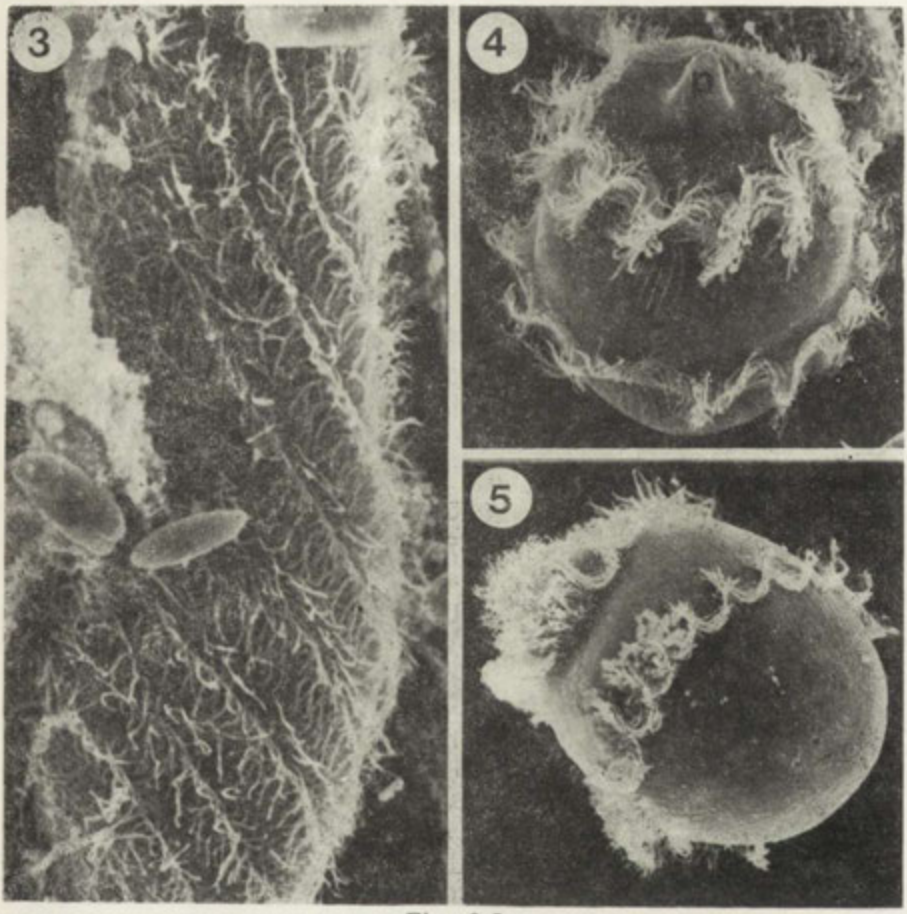
In the more active holotrich ciliates the cilia are quite closely spaced and beat at fairly high frequencies. As a result the cilia show clear metachronal waves. In *Paramecium* the whole body surface is covered by prominent dexioplectic metachronal waves (Fig. 3). In *Didinium* where the cilia are restricted to two narrow bands, the regular metachronal waves allow an easy discrimination between effective and recovery strokes of the beat (Fig. 4). In both cases the cilia can reverse their direction of beat, and show essentially similar but reversed dexioplectic wave patterns (e.g., Fig. 5). In less active holotrich ciliates, like *Tetrahymena*, the ciliary movement is less vigorous and the waves are less prominent, although the cilia still move in coordinated groups.

Heterotrich ciliates have both oral membranelles and somatic cilia. The latter are generally used for swimming, and form prominent waves in some cases (e.g., at the anterior end of *Blepharisma*) and less obvious coordinated groups in others. The membranelles may also contribute to swimming in some cases. It is interesting that the membranelles may continue to beat in the normal direction when the ciliate is swimming in reverse using its body cilia (at least in *Blepharisma*). While *Stentor* is being propelled backwards by its body cilia the membranelles may either be stationary or beating in reverse. It is suspected that the

Fig. 3. Scanning electron micrograph of *Paramecium* showing the extensive array of metachronal waves covering the surface. The effective stroke is from upper left to lower right (parallel to the wave crests) and the waves move from lower left to upper right (perpendicular to the wave crests). The anterior of the cell is at the top (× 1100). From Barlow and Sleigh (1979), J. Microsc., 115, p. 86

Figs. 4 and 5. Scanning electron micrographs of *Didinium*. In Fig. 4 the forward-swimming ciliate shows backwardly-directed effective strokes and cilia curving forwards (towards the proboscis, p) in their recovery strokes. Metachronal waves move from left to right. In Fig. 5 the backward-swimming ciliate shows effective strokes directed towards the proboscis (upper left) and metachronal waves that move from upper right to lower left (× 700)

Fig. 6. The use of cilia in feeding by *Vorticella*. The ciliary rows that comprise the polykinety (pk) and haplokinety (hk) form a ring around the margin of the peristome (c). Prominent waves of beating of polykinety cilia seen in top view (a) and in side view (b) pass towards the infundibulum, at the base of which is the cytostome. Beating of these cilia creates water currents that pass through the less active haplokinety cilia. The particles filtered from the current by the haplokinety are carried to the cytostome between the two rows of cilia. Modified from Sleigh and Barlow (1976)



Figs. 3–5

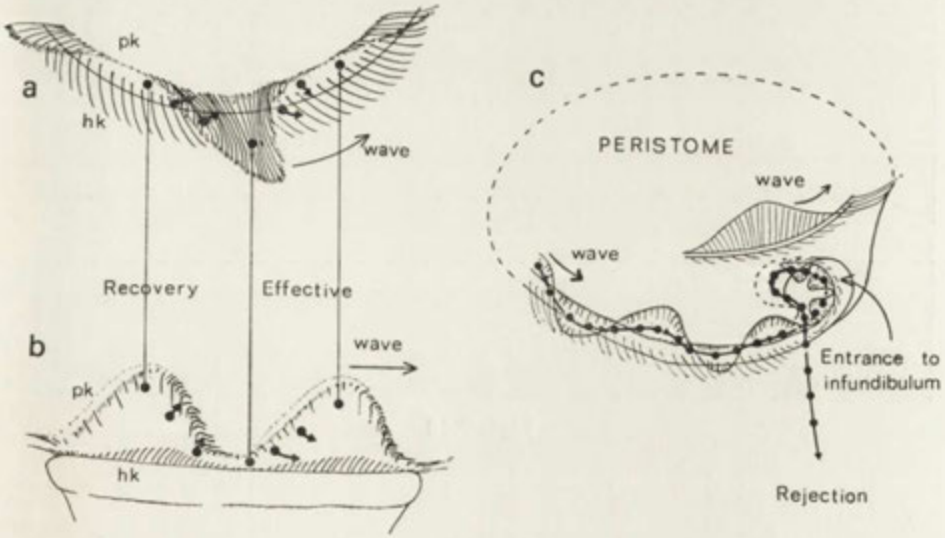
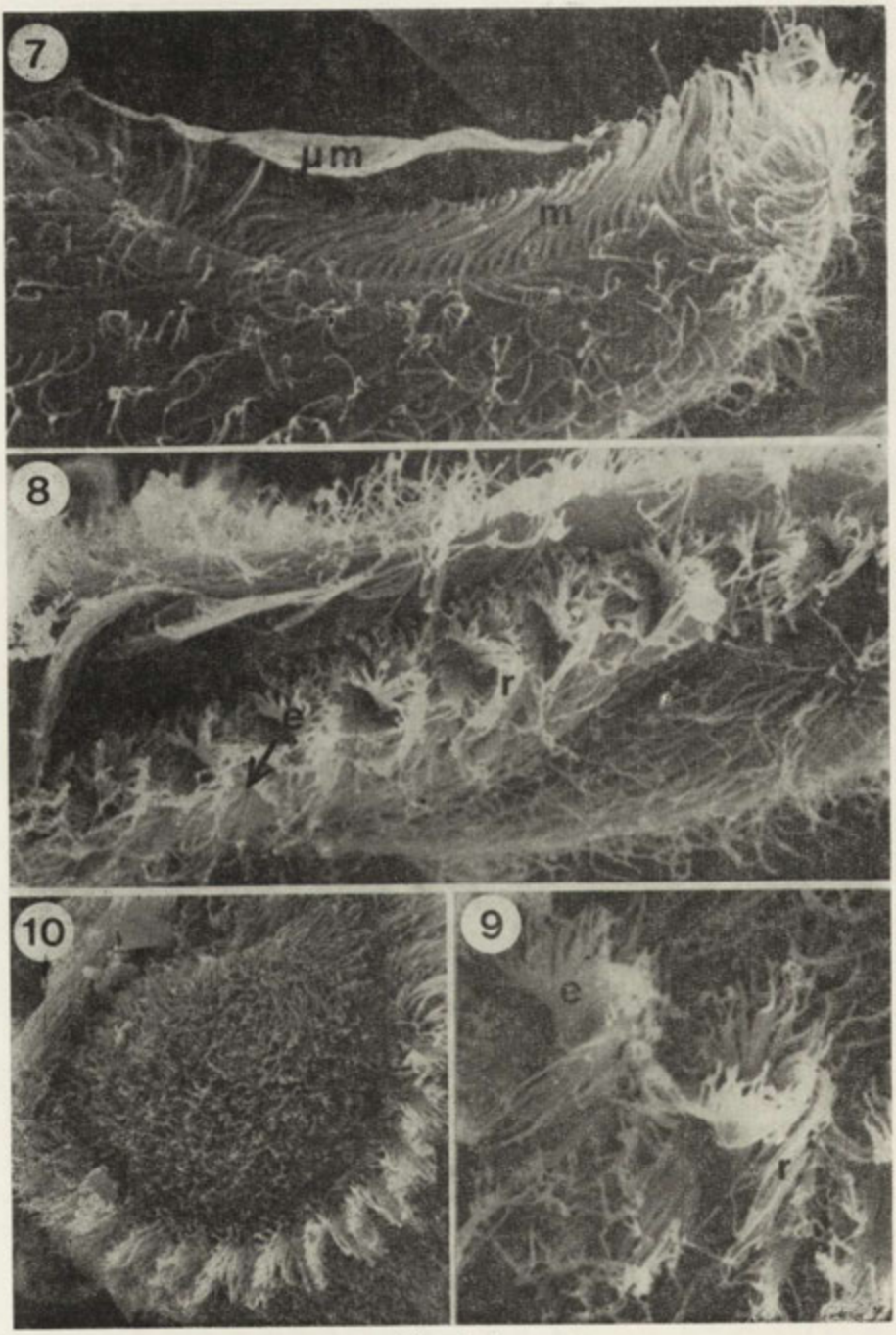


Fig. 6



Figs. 7–10

membranelles have a threshold for response to depolarization of the cell that is different from that of somatic cilia, a more intense stimulation being required to cause reversal of the membranellar beat.

Compound somatic cilia of hypotrichs and such oligotrichs as *Halteria* may be used in rapid swimming movements; where many cirri are present they may move in coordination (e.g., *Stylonychia* Machemer 1969).

Use of Cilia in Feeding by Ciliates

While many ciliates use cilia to swim in order to capture food by non-ciliary methods e.g., *Didinium*, this account will only be concerned with the use of cilia to create water currents from which food can be filtered, and with the use of cilia as filters. Two principal types of mechanism are used; in one type, exemplified by peritrichs like *Vorticella*, the two functions of current production and filtration are performed by different groups of cilia, whilst in heterotrichs like *Blepharisma* and *Stentor* the same cilia both propel the water currents and filter out the particles (see also Fenchel 1980).

The feeding of *Vorticella*, which we have previously described in some detail (Sleigh and Barlow 1976) makes use of rows of cilia which spiral up the infundibulum from the cytostome and continue around the rim of the cell body for over 360° (Fig. 6). A close-set, inner, double row of cilia forms the polykinety, the cilia of which beat metachronally in undulating waves which pass from the free end of the polykinety towards the cytostome. It their effective stroke these cilia move through about 90°, propelling water backwards all around the margin of the animal, and thereby creating the feeding current. In fact, these cilia perform the type of three dimensional motion described earlier, the recovery stroke moving to the left. The cilia of the single outer row form the haplokinety, the members of which are more

Figs. 7 and 8. Scanning electron micrographs of *Blepharisma* showing the membranelles (m) and undulating membrane (μm) from the left side of the animal (Fig. 7) and in ventral view (Fig. 8). In Fig. 7 most of the membranelles are inactive and the undulating membrane is held close to the membranelle tips. In Fig. 8 waves of activity are passing along the row of membranelles from the cytostome (at the left) towards the anterior (to the right); effective strokes (e) within these waves are seen as erect curved fans, whilst recovery strokes (r) are curved clumps of membranelles between these more open regions (× 1400). Fig. 7 is from Barlow and Sleigh (1979), J. Microsc., 115, p. 92

Figs. 9 and 10. Scanning electron micrographs of *Stentor*. The detail of active membranelles in Fig. 9 shows effective (e) and recovery (r) strokes comparable with those of *Blepharisma* in Fig. 8. (× 2500). In Fig. 10 the peristome of *Stentor* shows the ring of membranelles surrounding a field of small cilia which beat in a counter-clockwise direction towards the buccal area at the top left (× 500)

widely spaced (but still less than 1 μm apart) and form a filter which intercepts food particles and retains them between the haplokinety and polykinety. The cilia of the haplokinety beat with a small amplitude with waves approximately in phase with those of the polykinety. Particles caught between the two bands of cilia are carried between them towards the infundibulum by the movement of the metachronal waves and the asymmetrical beat of the cilia of the polykinety (Fig. 6).

The food collecting cilia of heterotrich ciliates are the membranelles of the adoral zone. The individual membranelles are formed from two or three close-set rows of closely packed cilia, with often 20 or more cilia per row, (Randall and Jackson 1958). The long axes of adjacent membranelle bases are parallel to one another and perpendicular to the main axis of the band of membranelles (Fig. 7). Waves of beating travel along the band of membranelles of the adoral zone from the cytostome towards the free end, each membranelle performing an effective stroke towards the right, as seen by an observer looking along the band from the cytostome towards the free end (Fig. 8). The component cilia of a membranelle do not beat in synchrony, but in a metachronal sequence, beginning with cilia at the oral (or inside) end of the rows and ending with those at the aboral (outer) end. As a result, the moving cilia of the membranelle form a curving fan that scoops water across the adoral zone during the effective stroke (Figs. 8, 9), before the cilia twist round in the usual counter-clockwise recovery stroke and become packed together to form an arched flattened bundle (Fig. 9). Several membranelles move close together as they curve back in their recovery strokes, but successive membranelles are widely separated in their effective strokes. The spacing between the membranelles is such that where one active propelling membranelle is at the middle of its effective stroke, the next is widely spread behind it at the start of the effective stroke and a third is behind that and approaching the end of its recovery stroke (Fig. 9). The water propelled by a membranelle in its effective stroke is drawn through a filter formed by the spreading component cilia of the one or two following cilia, which at that time are standing almost stationary. Food particles are therefore retained at the "upstream" side of the band of membranelles.

In *Blepharisma* the membranelles extend forwards from the cytostome along the (animal's) left side of the buccal area and an undulating membrane formed from a row of cilia along the right side of the mouth region arches over this buccal area (Fig. 7). The membranelles beat obliquely backwards (Fig. 8), and in doing so they draw water backwards through the tunnel formed under the undulating mebrane. Particles of a large range of sizes that are drawn through this tunnel

from the anterior end are retained in the buccal area by the 'filter-stage' of the membranelle cycle, and are carried back towards the cytostome by the water current being propelled by more posterior membranelles. Some food particles are taken into food vacuoles at the cytostome and others are released in a rejection stream between the most posterior membranelles and the undulating membrane at the rear of the buccal area.

In *Stentor* the membranellar band spirals out of the buccal area and around the margin of the broad end of the trumpet-shaped body (Fig. 10). Activity of the membranelles, whose counter-clockwise beat cycles include effective strokes moving at about 60–70° to the edge of the trumpet, draws water backwards from in front of the body and across the margin of the trumpet. Particles up to the size of rotifers filtered from this water current by the membranelles are retained within the membranelle ring at the edge of a peristomial field of small cilia (Fig. 10). The combined effects of the slightly oblique current flow and the activity of cilia of the peristomial field carry the retained particles around the edge of the peristomial field to the cytostome.

The cilia of protozoa are all believed to possess the same basic internal machinery. However, variation in their distribution, length and beat pattern (dependent upon modified use of the internal mechanism) allows them to perform a variety of different functions to high efficiency.

REFERENCES

Blake J. R. 1972: A model for the micro-structure in ciliated organisms. J. Fluid Mech., 55, 1–23.

Blake J. R. and Sleigh M. A. 1974: Mechanics of ciliary locomotion. Biol. Rev., 49, 85–125.

Fenchel T. 1980: Suspension feeding in ciliated protozoa: structure and function of feeding organelles. Arch. Protistenk., 123, 239–260.

Machemer H. 1969: Filmbildanalysen 4 verschiedener Schlagmuster der Marginalcirren von *Stylonychia*. Z. vergl. Physiologie, 62, 183–196.

Machemer H. 1974: Ciliary activity and metachronism in protozoa. In: Cilia and Flagella. (ed. M. A. Sleigh), Academic Press, London, 199–286.

Randall J. T. and Jackson S. F. 1958: Fine structure and function in *Stentor polymorphus*. J. biophys. biochem. Cytol., 4, 807–830.

Sleigh M. A. 1976: Fluid propulsion by cilia and the physiology of ciliary systems. In: Perspectives in Experimental Biology, Vol. 1, Zoology. (ed. P. Spencer Davies), Pergamon Press, Oxford, 125–134.

Sleigh M. A. 1981: Flagellar beat patterns and their possible evolution, Biosystems, (in press).

Sleigh M. A. and Aiello E. 1972: The movement of water by cilia. Acta Protozool., 11, 265–277.

Sleigh M. A. and Barlow D. I. 1976: Collection of food by *Vorticella*. Trans. Am. Microsc. Soc., 95, 482–486.

Sleigh M. A. and Barlow D. I. 1980: Metachronism and control of locomotion in animals with many propulsive structures. In: Aspects of Animal Movement. (eds. H. Y. Elder and E. R. Trueman), Cambridge University Press, Cambridge, 49–70.

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Motility and Behaviour: Contributed Paper Session in Memory of Professor Theodore L. Jahn

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Co-chairman and Convener: Stanisław Dryl, Nencki Institute of Experimental Biology, Warszawa, Poland

Preface

Theodore L. Jahn was a widely recognized and highly esteemed investigator, thinker and educator. He published many papers and articles concerning the physiology and biophysics of protozoa and other themes, including systematics. In the popular book "How to know the *Protozoa*" (1949), written jointly with Frances F. Jahn, he gave an adequate explanation of what the protozoa are and what their positions are in the living world. His greatest and the most enduring influence is, however, his concept of the protozoan motile behaviour.

Professor Jahn's ideas were recalled and reconstructed in short presentations by Cicily Chapman-Andresen, Chairperson, and Stanisław Dryl, Convener, of the Session.

Excitability and Motor Responses in Ciliates

Mechanical stimuli to the cell posterior ends of ciliates elicit hyperpolarizing receptor potentials due to a conductance increase of the membrane to K^+ ions (Naitoh and Eckert 1973).

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These receptor responses were investigated in *Stylonychia mytilus* under voltage-clamp conditions using two intracellular microelectrodes by Deitmer (1981), who showed that changes in the mode of mechanical stimulation influence the amplitude of the mechanoreceptor current, which, in turn, affects the time course of the receptor current. The reversal potential of the mechanoreceptor current is 88 mV at $(K)_0$ of I mM, and varies with $(K)_0$ according to the Nernst equation. Thus, the reversal potential is identical with K^+, the equilibrium potential.

The receptor current-voltage relationship is linear in the hyperpolarizing direction, but markedly non-linear in the depolarizing direction. The exponential decay of the receptor current is also changed with the membrane potential, its time constant increasing e-fold with a hyperpolarization of 110 mV up to the reversal potential. Beyond the reversal potential the time constant of decay appeared considerably reduced for small receptor inward currents before increasing again with further hyperpolarization. On the basis of this data, Deitmer (1981) suggested that *Stylonychia* may serve as a model for studying the mechano-electrical transduction process at a cellular level, since the liftetime of the mechanically activated ionic channels is influenced by the membrane potential, as reported for chemically activated channels in the postsynaptic membrane.

Janiszewski and Kurdybacha (1981) reported results support Hildebrand and Dryl's (1976) hypothesis on inactivation of the membrane voltage-sensitive calcium channels of *Paramecium caudatum* by intracellular calcium.

The data presented by Fabczak (1981) showed the multiionic nature of the membrane potential differences in *Stentor coeruleus*. Lowering of the temperature from 18°C do 8°C causes a regular and reproducible hyperpolarization of the plasmalemma and an increase of the input resistance. Fabczak (1981) stated that the ionic permeability ratio $\alpha = (P_{Na}/P_K/)$ decreases continuously with a lowering of the temperature within the entire tested range. Analysis of the changes in resistance suggests distinctly, that P_{Na} is more affected than P_K when temperature is lowered.

The "lateral" doublets of *Stylonychia mytilus* show oblique cathodal responses (OCR) of the SE–NW direction while the so-called "dorsal" doublets swim directly towards the cathode parallell to the applied DC field. According to Dryl et al. (1981), the differences in the behaviour of two doublet forms exposed to electric DC field can be explained by the fact that the "lateral" asymmetrical doublets are strongly thigmotactic while the "dorsal" doublets are highly motile,

swimming organisms with reduced thigmotactic properties. It is suggested that OCR can be shown only by thigmotactic ciliates as a result of morphological asymmetry and functional differences of locomotor organelles in two cellular components.

Ciliary and Flagellar Reorientation

In ciliates the cilia perform two functions — those of propulsing and steering (Kuźnicki 1970). The most extensively studied steering function is ciliary reversal in *Paramecium*. Several authors suggests that ciliary reversal is one of the adaptive differentiations of the ciliary function, essential for behavioural responses in ciliates. In addition to its primary function of propelling fluids over the cell, the euglenoid flagellum, like the *Paramecium* cilia, performs a steering function. In *Euglena* the strophophobic response shows a 90° change in the flagellum orientation from normal trailing to a position perpendicular to the long axis of the cell. The forward swimming ceases and the cell begins pivoting (Diehn 1979, Diehn et al. 1977, Mikołajczyk and Diehn 1976). Some new data on *Euglena* phobic responses (Mikołajczyk and Kuźnicki (1981) allowed Kuźnicki (1981) to put foreward a hypothesis that there are phylogenetic relation and a functional relation between these patterns of ciliary and flagellar motility. This problem can only be a matter for speculation at present. However, it would be an attractive idea to postulate that the same molecular mechanism affects flagellar reorientation in *Euglena* during phobic responses and the reversal of the beat direction in the cilia of *Paramecium*.

In *Paramecium*, the reorientation of the cilia from the normal to the reversal position may occur in beating, as well as in non-beating cilia (Grębecki and Mikołajczyk 1968, Kuźnicki 1963, Naitoh 1966). The separation of both functions can be experimentally evoked in *Euglena*. It means that the ciliary and flagellar function, that is propelling and steering, can operate independently. There is a considerable number of data indicating that two contractile mechanisms operate in the *Paramecium* ciliary system (Kuźnicki 1970, Naitoh 1969, Naitoh and Eckert 1974). One of them is concerned with cyclic bending of calcium while the other accounts for the direction of the beating. Both contractile systems are energized by ATP, but the ciliary reversal alone employs Ca^{2+} ions for ATPase activites.

Since Hoffmann-Berling (1955) introduced the technique of glycerination, it has been well known, that the glycerol or detergent models of ciliates and flagellates are reactivated and swim forward in

the solution of ATP + Mg^{2+} with EGTA (see Arronet 1973 and Naitoh and Eckert 1974 for detailed references). The ciliary reversal of non-beating (Naitoh 1968, 1969) and beating cilia of *Paramecium* models (Naitoh and Kaneko 1972) occurs in the presence of ATP and Ca^{2+} ions in a concentration above 10^{-6} M. It also appears that there are similar motile components in the flagella of *Euglena* and *Chlamydomonas* (Hyams and Borisy 1975, Nichols and Rikmenspoel 1977, 1978, Schmidt and Eckert 1976), but the ionic activation mechanism is not as clear as in *Paramecium*. In living specimens, Mg^{2+} + ATP is required to maintain the cyclic bending of a flagellum. An increase of internal Mg^{2+} stimulates flagellar beating, while the microinjection of Ca^{2+} ions inhibits the flagellar motility (Nichols and Rikmenspoel 1978).

Calmodulin, an important regulatory component of a number of molecular mechanisms, was recorded in *Euglena gracilis* (Kuźnicki et al. 1979), *Paramecium* and *Tetrahymena* cilia (Maihle and Satir 1980). The calmodulin complex may be part of an axonemal switch that evokes ciliary stoppage in ciliated epithelial cells (Reed and Satir 1980) and a ciliary reversal in protozoa (Satir et al. 1980). A similar calmodulin-mediated molecular mechanism may also operate in *Euglena* flagella (Kuźnicki 1981).

In conclusion it is suggested that reorientation of the *Euglena* flagellum, the ciliary reversal in *Paramecium* and ciliary arrest of ciliated epithelial cells are all elicited by the same mechanism which appeared early in phylogeny and was already operative in primitive flagellates.

Photoreception and Transduction Chains in Flagellates

Since the beginning of this century scientists have been fascinated by the problems of photobehaviour of photosynthesizing green flagellates. There were two major lines of study: the first concerned with the physiological aspects of photomovement and the second, trying to solve the problem of photoreception of the light stimulus and transduction of the received signal through the processor to the effector-flagellum.

The recent few years show a very significant contribution to our knowledge of the nature of the photoreceptor and the process of phototransduction as well as of the ionic background of the relation between the photoreceptor pigment and the induction of flagellar reorientation (Colombetti and Lenci 1979, Omodeo 1979, Banchetti et al. 1980, Diehn 1980, Doughty and Diehn 1980a, b, Doughty et al. 1980). However, many aspects of this crucial problem are

still to be solved. For example it is very important to determine the number of photoreceptor systems in *Euglena*. There may be only one such system responsible for both photophobic responses — step-up (caused by an increase in light intensity above the threshold) and step-down (caused by a decrease in light intensity), or two — one for the step-up and the other for the step-down response — as postulated by Diehn (1972) and Mikołajczyk and Diehn (1975). If we assume that *Euglena* has two photoreceptors, we are faced by the problem of their localization. At present we have ample evidence that the photoreceptor responsible for the step-down photophobic response is the paraflagellar swelling (paraflagellar body) located within the locomotor flagellum, near its base. Where should one look for the photoreceptor engaged in the step-up response? All the data available point to flavins as a photoreceptor pigment for the step-down response (Colombetti and Lenci 1979, Omodeo 1979, Doughty and Diehn 1980 a). Until recently it was uncertain whether the same pigment was also responsible for eliciting of the step-up response. In the last few years some amounts of flavins have been found within the stigmatal pigments (Sperling-Pagni et al. 1976), so an active role for the stigma in photoresponses, for example as a second photoreceptor cannot be ruled out.

It was commonly accepted that the stigma, which contains mostly carotenoids (Batra and Tollin 1964, Bartlett et al. 1972, Heelis et al. 1979, 1980) plays a passive role as a screen for the real photoreceptor, the paraflagellar body (Diehn 1969 a, 1973, Lenci and Colombetti 1978 and others). Carotenoids alone are unlikely to function as a photoreceptor pigment in *Euglena* (Song et al. 1972, Song and Moore, 1974, Heelis et al. 1981). The presence of stigma is, however, required for photoaccumulation (Diehn and Tollin, 1966, Checcucci et al. 1976, Ferrara and Banchetti 1976). It is well known that *Astasia*, deprived of both the stigma and the paraflagellar body does not respond to light while a streptomycin-bleached *Euglena*, with its stigma pigmentation removed, can only exhibit a step-up photophobic response.

The paper presented by Francesco Lenci (Colombetti et al. 1981) addressed to the question of whether *Euglena* has one or two separate photosensory transduction chains. The authors studied the ability of *Euglena gracilis* to exhibit photophobic responses, depending on the growth stage of the green, dark-grown or streptomycin-bleached cells. They have shown, that there is a close correlation between the ability of the cells to respond to a decrease (step-down) or an increase (step-up) in light intensity and the age of the green culture. At the lag phase no step-down response was observed whereas the step-up response was

quite conspicuous. However, as the culture aged, the percentage of cells showing the step-down photophobic response increased, while, at the same light intensity, the percentage of cells exhibiting the step-up response decreased. According to Colombetti et al. (1981) these results point to the possibility that in the lag phase the photosensory system of the step-down response probably does not operate, manifesting itself only later. In the case of the step-up response, the photosensory system operates both in the lag and in the stationary phase of the culture. In the stationary phase however, the threshold for the step-up response becomes higher. The experiments with dark-bleached cells in the stationary phase show that the cells are capable of both the step-down and step-up responses at the light intensity which evokes nothing but the step-down response in green cultures. The streptomycin-bleached cells, as expected, elicited the step-up response only, what would suggest a complete lack of a photosensory system for the step-down response. These results could, according to Colombetti et al. (1981), indicate to the presence of two separate sensory transduction chains. The comparison of the action spectra of the streptomycin-bleached cells with those of the green cells (Diehn 1969 b, Barghigiani et al. 1979, Checcuci et al. 1976). allowed the conclusion that flavins are the photoreceptor pigment for both photophobic responses.

The disappearance and reappearance of the photophobic responses depend not only on the age of the culture and the presence or absence of stigma and paraflagellar body, but are also seen in *Euglena* transferred to a buffered solution containing calcium, magnesium and potassium ions (Mikołajczyk and Pado 1981). Mikołajczyk and Pado (1981) reported that in rinsed cells the disappearance of the step-down response was simultaneous with a decrease in the threshold for the step-up response. This means that the step-up response appears at the light intensity usually associated with the step-down response. The step-down response gradually reappears a few hours later simultaneously with an increase in the threshold for the step-up response. The disappearance of the step-down response in rinsed cells was earlier observed by Doughty and Diehn (1980 b). The molecular mechanism of the disappearance and reappearance of the photophobic responses is unknown awaiting an answer in the future.

Ristori et al. (1981) using electrophysiological methods tried to localize of the photoreceptor and the active membrane in the green alga *Haematococcus pluvialis*. The authors recorded a resting potential, and an extracellular photoinduced electric potential regenerated by a 2 μm beam of light (within the blue or green range of the spectrum) focused on the stigma. The obtained results suggest that the photoreceptor pigment as well as an active membrane are located in the stigma region of the cell.

Although the results presented in this Contributed Paper Session come from different research fields they share a common interest in protozoan motile behaviour. During the last four years an important progress had been made in the understanding of the photobehaviour of flagellates. Recently it became clear that there is not only a structural but also a functional uniformity between cilia and flagella. This is a hopeful prospect for future studies of the motile behaviour of *Protozoa*.

REFERENCES

Arronet N. J. 1973: Motile muscle and cell models. Consultants bureau. New York-London, 192.

Banchetti R., Rosati G. and Verni F. 1980: Cytochemical analysis of the photoreceptor in *Euglena gracilis* Klebs (*Flagellata Euglenoidina*). Monit. zool. ital. (N.S.), 14, 165–171.

Barghigiani C., Colombetti G., Francinni B. and Lenci F. 1979: Photobehavior of *Euglena gracilis* — actin spectrum for the step-down photophobic response of individual cells. Photochem. Photobiol., 29, 1015–1019.

Bartlett C. J., Walne P. L., Schwarz O. J. and Brown D. M. 1972: Large scale isolation and purification of eyespot granules from *Euglena gracilis* var. *bacillaris*. Plant Physiol., 49, 881–885.

Batra P. P. and Tollin G. 1964: Phototaxis in *Euglena* I. Isolation of the eye-spot granules and identification of the eye-spot pigments. Biochim. Biophys. Acta, 79, 371–378.

Checcucci A., Colombetti G., Ferrara R., Lenci F. 1976: Action spectra for photoaccumulation of green and colorless *Euglena*; evidence for identification of receptor pigments. Photochem. Photobiol., 23, 51–54.

Colombetti G. and Lenci F. 1979: Identification and spectroscopic characterization of photoreceptor pigments. In: Photoreception and Sensory Transduction in Aneural Organisms. (F. Lenci and G. Colombetti eds.). Plenum Press, New York and London, 173–188.

Colombetti G., Lenci F., Passarelli V., Quaglia M. and Rinaldi-Fornaca G. 1981: Are there two separate photosensory transduction chains in *Euglena*? In: Progress in Protozool. Abstr. Sixth Int. Congr. Protozool., Warszawa, 61.

Deitmer J. W. 1981: Voltage-clamp analysis of a mechanoreceptor response in the hypotriche ciliate *Stylonychia mytilus*. In: Progress in Protozoology, Abstr. Sixth Int. Congr. Protozool., Warszawa, 71.

Diehn B. 1969 a: Phototactic response of *Euglena* to single and repetitive pulses of actinic light: orientation time and mechanism. Expl. Cell Res., 56, 375–381.

Diehn B. 1969 b: Action spectra of the phototactic responses in *Euglena*. Biochim. Biophys. Acta, 177, 136–143.

Diehn B. 1972: The receptor–effector system of phototaxis in *Euglena*. Acta Protozool., 11, 325–332.

Diehn B. 1973: Phototaxis and sensory transduction in *Euglena*. Science, 181, 1009–1015.

Diehn B. 1979: The interactions of photic and chemical stimulus response systems in *Euglena gracilis*. Acta Protozool., 18, 7–16.

Diehn B. 1980: Photomovement and photosensory transduction in microorganisms. Photochem. Photobiol., 31, 641–644.

Diehn B. and Tollin G. 1966: Phototaxis in *Euglena* III. Lag phenomena and the overall mechanism of the tactic response to the light. Photochem. Photobiol., 5, 839–844.

Diehn B., Feinleib M., Haupt W., Hildebrand E., Lenci F. and Nultsch W. 1977: Terminology of behavioral responses in motile microorganisms. Photochem. Photobiol., 26, 559–560.

Doughty M. J. and Diehn B. 1980a: Flavins as photoreceptor pigments for behavioral responses in motile microorganisms, especially in the flagellated alga, *Euglena* sp. Struct. Bonding, 41, 45–70.

Doughty M. J. and Diehn B. 1980b: Photosensory transduction in the flagellated alga, *Euglena gracilis*. I. Action of divalent cations, Ca^{2+} antagonists and Ca-ionophore on motility and photobehaviour. Biochim. Biophys. Acta, 588, 148–168.

Doughty M. J., Grieser R. and Diehn B. 1980: Photosensory transduction in the flagellated alga, *Euglena gracilis*. 2. Evidence that blue-light effects alteration in Na^+–K^+ permeability of the photoreceptor membrane. Biochim. Biophys. Acta, 601, 10–23.

Dryl S., Kubalski A. and Totwen-Nowakowska I. 1981: Galvanotactic responses of *Stylonychia mytilus* doublets. In: Progress in Protozoology, Abstr. Sixth Int. Congr. Protozool., Warszawa, 84.

Fabczak S. 1981: Electrophysiological characteristics of the cell membrane of *Stentor*. In: Progress in Protozoology, Abstr. Sixth Inter. Congr. Protozool., Warszawa, 91.

Ferrara R. and Banchetti R. 1976: Effect of streptomycin on the structure and function of the photoreceptor apparatus of *Euglena gracilis*. J. Expl., Zool., 198, 393–402.

Grębecki A. and Mikołajczyk E. 1968: Ciliary reversal and re-normalization in *Paramecium caudatum* immobilized by Ni ions. Acta Protozool., 5, 297–304.

Heelis D. V., Kernick W., Phillips G. O. and Davies K. 1979: Separation and identification of the carotenoid pigments isolated from light-grown cells of *Euglena gracilis* strain Z. Arch. Mikrobiol., 121, 207–211.

Heelis D. V., Heelis P. F., Kernick W. A. and Phillips G. O. 1980: The stigma of *Euglena gracilis* strain Z: an investigation into the possible occurrence of carotenoproteins and nucleic acids. Cytobios, 29, 135–143.

Heelis D. V., Heelis P. F., Bradshow F. and Philips G. O. 1981: Does the stigma of *Euglena gracilis* play an active role in the photoreception processes of this organism? A photochemical investigation of isolated stigma. Photochem. Photobiophys., 3, 77–82.

Hildebrand E. and Dryl S. 1976: Significance of Ca^{2+} and K^+ ions for the excitation of the protozoan membrane. Bioelectrochem. Bioenerg., 3, 543–544.

Hoffmann-Berling H. 1955: Geisselmodelle und Adenosintriphosphat (ATP). Biochim. Biophys. Acta, 16, 146–154.

Hyams J. S. and Borisy G. G. 1975: The dependence of the waveform and direction of beat of *Chlamydomonas* flagella on calcium ions. J. Cell Biol., 67, 186a.

Jahn T. L. and Jahn F. F. 1949: How to know the protozoa. WM. C. Brown Company, Dubuque, Iowa.

Janiszewski J. and Kurdybacha J. 1981: Effects of various levels of external pH and pCa on barium-induced excitation and motor response of *Paramecium caudatum*. In: Progress in Protozoology, Abstr. Sixth Int. Congr. Protozool., Warszawa, 153.

Kuźnicki L. 1963: Reversible immobilization of *Paramecium caudatum* evoked by nickel ions. Acta Protozool., 1, 301–312.

Kuźnicki L. 1970: Mechanism of the motor responses of *Paramecium*. Acta Protozool., 8, 83–118.

Kuźnicki L. 1981: Comparison between the ciliary reversal of *Paramecium* and the rapid reorientation in flagellum position of *Euglena*. In: Progress in Protozoology, Abstr. Sixth Int. Congr. Protozool., Warszawa, 208.

Kuźnicki J., Kuźnicki L. and Drabikowski W. 1979: Ca^{2+}-binding modulator protein in protozoa and myxomycete. Cell Biol. Int. Rep., 3, 17–23.

Lenci F. and Colombetti G. 1978: Photobehaviour of microorganisms: a biophysical approach. Ann. Rev. Biophys. Bioeng., 7, 347–361.

Maihle N. J. and Satir B. H. 1980: Calmodulin in the ciliates *Paramecium tetraurelia* and *Tetrahymena thermophila*. In: Calmodulin and Cell Function. (ed. D. M. Watterson and F. F. Vincenzi), Ann. New York Ac. Sci., 356, 408–409.

Mikołajczyk E. and Diehn B. 1975: The effect of potassium iodide on photophobic responses in *Euglena*: Evidence for two photoreceptor pigments. Photochem. Photobiol., 22, 269–271.

Mikołajczyk E. and Diehn B. 1976: Light-induced body movement of *Euglena gracilis* coupled to flagellar photophobic response by mechanical stimulation. J. Protozool., 23, 144–147.
Mikołajczyk E. and Kuźnicki L. 1981: Body contraction and ultrastructure of *Euglena*. Acta Protozool., 20, 1–24.
Mikołajczyk E. and Pado R. 1981: The kinetics of photobehavior modification upon change of the suspension medium of *Euglena gracilis*. In: Progress in Protozoology, Abstr. Sixth Int. Congr. Protozool., Warszawa, 253.
Naitoh Y. 1966: Reversal response elicited in nonbeating cilia of *Paramecium* by membrane depolarization. Science, 154, 660–662.
Naitoh Y. 1968: Ionic control of the reversal response of cilia in *Paramecium caudatum*: A calcium hypothesis. J. gen. Physiol., 51, 85–103.
Naitoh Y. 1969: Control of the orientation of cilia by adenosine triphosphate, calcium and zinc in glycerol-extracted *Paramecium caudatum*. J. gen. Physiol., 53, 517–529.
Naitoh Y. and Eckert R. 1973: Sensory mechanisms in *Paramecium*. II. Ionic basis of the hyperpolarizing mechanoreceptor potential. J. Expl. Biol., 59, 53–65.
Naitoh Y. and Eckert R. 1974: The control of ciliary activity in *Protozoa*. In: Cilia and Flagella (M. A. Sleigh ed.). New York and London, Academic Press, 305–352.
Naitoh Y. and Kaneko H. 1972: ATP-Mg-reactivated Triton-extracted models of *Paramecium*: Modification of ciliary movement by calcium ions. Science, 176, 523–524.
Nichols K. M. and Rikmenspoel R. 1977: Mg^{2+}-dependent electrical control of flagellar activity in *Euglena*. J. Cell Sci., 23, 211–225.
Nichols K. M. and Rikmenspoel R. 1978: Control of flagellar motion in *Chlamydomonas* and *Euglena* by mechanical microinjection of Mg^{2+} and Ca^{2+} and by electric current injection. J. Cell Sci., 29, 233–247.
Omodeo P. 1979: The photoreceptive apparatus of flagellated algal cells: Comparative morphology and some hypothesis on functioning. In: Photoreception and Sensory Transduction in Aneural Organisms. (F. Lenci and G. Colombetti eds.). Plenum Press, New York and London 127–153.
Reed W. and Satir P. 1980: Calmodulin in mussel gill epithelial cells: role in ciliary arrest. In: Calmodulin and Cell Functions. (D. M. Watterson and F. F. Vincenzi eds.), Ann. New York Acad. Sci., 356, 423–426.
Ristori T., Ascoli C., Banchetti R., Parrini P. and Petracchi D. 1981: Localization of photoreceptor and active membrane in the green alga *Haematococcus pluvialis*. In: Progress in Protozoology, Abstr. Sixth Int. Congr. Protozool., Warszawa, 314.
Satir P. 1979: Regulation of microtubule sliding in cilia. Acta Protozool., 18, 25–31.
Satir B. H., Garofalo R. S., Giligan D. M. and Maihle N. J. 1980: Possible functions of calmodulin in protozoa. In: Calmodulin and Cell Functions. (D. M. Watterson and F. F. Vincenzi eds.), Ann. New York Acad. Sci., 356, 83–91.
Schmidt J. A. and Eckert R. 1976: Calcium couples flagellar reversal to photostimulation in *Chlamydomonas reinhardii*. Nature, London, 262, 713–715.
Song P. S., Moore T. A. and Sun M. 1972: Excited states of some plant pigments. Adv. Food Res. Supp., 3, 33–74.
Song P. S. and Moore T. A. 1974: On the photoreceptor pigment for photoropism and phototaxis: is a carotenoid the most likely candidate? Photochem. Photobiol., 19, 435–441.
Sperling-Pagni P. G., Walne P. L. and Wehry E. L. 1976: Fluorimetric evidence for flavins in isolated eyespots of *Euglena gracilis* var. *bacillaris*. Photochem. Photobiol., 24, 373–375.

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Genetics and Development: Contributed Paper Session in Memory of Professor Tracy M. Sonneborn

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Chairman: David L. Nanney, University of Illinois, Urbana, USA
Co-Chairman: Gerard deHaller, Universite de Geneve, Geneve, Switzerland
Joseph Frankel, University of Iowa, Iowa City, USA
Renzo Nobili, Istituto di Zoologia, Pisa, Italy
Convenor: Maria Jerka-Dziadosz, Nencki Institute of Experimental Biology, Warszawa, Poland

This session began with a commentary by D. L. Nanney on the life and work of T. M. Sonneborn. The twenty two contributed papers following were concerned with many of the issues addressed by Sonneborn's work; cytology, genetics, mating behavior, morphogenesis. Several of the participants (Nanney, Heckmann, de-Haller, Nobili, Inoki, Bleyman, Miyake) had spent time in Sonneborn's laboratory in Bloomington, and many more had strong professional and personal ties with him.

Most of the contributed papers represented work on ciliates, but two dealt with trypanosomes. S. Inoki (Kashihara with H. Osaki and M. Furuya) reported microfluorimetric studies of DNA in single cells of *T. gambiense* and *T. cruzi*. The techniques showed distinctive differences in the amounts of nuclear DNA in cells of the two strains, and systematic differences in the relative amounts of kinetoplastic DNA. The other trypanosome paper was presented by C. J. Shinondo from the University of Zambia. Shinondo's paper described the effects of colchicine and vinblastine on morphogenesis *in vitro*. At 5×10^{-3} and 1×10^{-3} M., colchicine inhibited cell division and pro-

Session took place on July 10 at VI International Congress of Protozoology, Warszawa, Poland, 5–11 July 1981.

duced aberrant cells. Vinblastine sulfate also inhibited division, but had no obvious effect on morphogenesis. The effects of both drugs were reversible. Their modes of action in this case are uncertain because of their very low uptake by the cells.

Six of the 22 papers reported work on some species of *Paramecium*. Stephen F. Ng (Hong Kong) reported the consequences of introducing by microinjection genetically marked micronuclei into cells of *P. tetraurelia* that had been rendered amicronucleate with a laser microbeam. Some of the introduced micronuclei were non-functional, i.e., were unable to yield normal nuclear equipment at a subsequent reorganization. Others gave unexpected results in competition with resident micronuclei; some were at a selected advantage, and some were strongly selected against. The normal behavior of most of the transplanted nuclei, however, shows the potential utility of this technique in genetic studies. The value of microinjection was also shown in the paper by H. Kościuszko (Kraków, with S. A. Koizumi, Sendai). This study showed that clonally aged macronuclear material of *P. tetraurelia* injected into young cells caused autogamy to occur in them. The reciprocal injections were without effect.

Three papers on *Paramecium primaurelia* were contributed by the workers at the University of Genoa: M. U. Delmonte-Corrado, P. Ramoino, E. Margallo and T. Crippa-Franceschi in one paper; P. Ramoino, M. U. Delmonte-Corrado, C. Calvi-Parisetti and T. Crippa-Franceschi in another. A third paper on "Clonal development and a kind of phenotypic change in *P. primaurelia*" was inserted into the program late, and the abstract was distributed at the meeting. All these studies are concerned with covariation in various biological characteristics- with temperature sensitivity, DNA content, clonal age, vegetative pedigrees, mating types and growth mode. They demonstrate a variety of statistical relationships with uncertain mechanisms of association. One of the more interesting relationships is between the postzygotic growth mode and mating types. According to Crippa-Franceschi, caryonides destined to manifest mating type I show a regular growth pattern after autogamy or conjugation, but caryonides that will subsequently show themselves as type II grow irregularly; they divide at first very slowly and then catch up in a burst of rapid divisions. This curious correlation merits further study.

The sixth *Paramecium* paper was presented by M. E. Kawulok (Hawaii, with R. D. Allen), and described the complex morphogenetic events associated with the development of the cytostome-cytoproct complex in *P. caudatum*.

The six *Tetrahymena* papers also covered a wide range of experimental areas. The most genetic of the studies was reported in a paper submitted late by L. K. Bleyman Baruch College, New York, with E. Orias, M. Baum and P. J. Bruns) and illustrated the genetic engineering technology described in more general terms in a plenary session by Bruns. Using nullisomic stocks for chromosomal mapping, Bleyman and colleagues found the *Mat* locus (controlling mating type potentialities) is located on the second of the five *Tetrahymena* chromosomes.

Three other *Tetrahymena* studies dealt with characteristics of particular mutants of *T. thermophila*. G. Cleffman (Justus-Liebig University, with E. Schäfer) reported growth studies on the mutant *conical*, which divides its cytoplasm unequally at each cell division. Earlier studies (Doerder et al.) had shown that the smaller daughters have a much extended cell cycle. The new results show that the rate of growth of the cells is proportional to the cell size; the small cells grow proportionately more slowly than do the large cells. Thus, equality of macronuclear chromatin does not assure equality of synthetic activity. Size compensation is made more difficult by this size related inequality of growth rate; cell cycle length is adjusted to permit eventual approximate equality of cell size.

J. Frankel (Iowa City, with M. Nelsen) presented an analysis of the onset of the pattern change in the mutant *janus*, that produces a partial mirror-image doublet when it is homozygous. The analysis of phenomic lag in *Janus* suggests that the mutation's primary effect is not to alter the system of reference structures for the assessment of positional information, but rather to alter the cell's responses to constant reference structures.

A. Kaczanowski (Warsaw) reported studies of the effects of cycloheximide on cycloheximide resistant and sensitive cells during conjugation in *T. thermophila*. The effects of cycloheximide on sensitive cells depend upon the time of exposure; early exposure aborts meiosis and yields a curious and possibly useful elongated metaphase nucleus. After fertilization, treatment results in abortion of macronuclear development; heterokaryons are formed with the old macronuclei persisting. Doses of cycloheximide capable of aborting sensitive conjugation do not abort mixed pairs (one sensitive and one resistant), though conjugation appears to be slightly delayed in both cells.

J. Bąkowska (Warsaw, with E. M. Nelsen and N. E. Williams, Iowa City) in a communication presented by J. Frankel, analyzed the effects of rapid starvation on the structure of the oral apparatus. Their preparations, following a technique by Vaudaux and visualization by scanning scope, elegantly display the bases of the

membranelles. Starved cells show a substantial reduction in the number of basal bodies, but show a strict conservation of the pattern of the residual structures.

J. Wolfe (Wesleyan University, Middletown, CT) reported studies on changes associated with co-stimulation in *T. thermophila*. In particular, he reported that protein synthesis is altered quantitatively when appropriate mixtures of different mating types are made. A protein celled p80 (80k daltons) is apparently produced at a much higher rate in co-stimulated cells.

Two papers were presented on *Blepharisma*, both concerned with the induction of meiosis. K. Heckmann (Münster, with E. Friedel and A. Miyake) presented a systematic analysis of the effects of cycloheximide on the progress of meiosis. They show that cycloheximide halts meiosis at one of six different stages when it is added at progressively later stages. These effects are reversible for a time; if the cells are washed within 8 h of treatment, they will resume their interrupted affairs. The paper by G. Santangelo (Pisa, with R. Nobili) was concerned with the initiation of meiosis rather than its progress. These workers used homotypic (non-inducing) and heterotypic (inducing) unions and surgical separation to explore further the properties of the inducing substance previously reported by Miyake. They show that an activated cell can only rarely transfer activation to a homotypic pair after being separated from its heterotypic stimulus. They also show that the spread of induction in homotypic chains (after separation from the heterotypic inducer) is reduced in the presence of cycloheximide. These studies suggest that the meiotic inducer may require protein synthesis in order to be transmitted along a chain.

Two papers were concerned with mating in hypotrich ciliates, N. Ricci (Pisa) continues his ethographic analysis of sexual behavior in *Oxytricha bifaria*, particularly attempting to relate the mating maneuvers in this species to the organisms' general exploratory behavior. He shows that the couples mating steps can be interpreted as modifications of their scanning behavior, and documents the progressive gain and loss of the focussed response when a heterologous stimulus is supplied and removed. In the other hypotrich paper, P. Luporini (Pisa, with R. Dallai), provided an ultrastructural analysis of events at the mating surfaces of *Euplotes crassus*.

Finally, four organisms were represented by a single presentation each. I. B. Raikov (Leningrad) described the nuclear apparatus of *Geleia orbis*. This karyorelictida species has non-dividing "paradiploid" macronuclei that are assorted and developed a new at each cell division. The macronuclei contain various ultrastructural components of uncertain function, but also some reasonably identified nucleolar material. The

total DNA varies from about 4N to 20N. The variable DNA content probably reflects in part a differential amplification of the ribosomal DNA and perhaps of other cistrons.

M. Suhama (Hiroshima) presented evidence that the dividing micronucleus in *Glaucoma scintillans* is located regularly with respect to the cortical structures; all are observed within a restricted region on the right dorsal region of the cell. Mirror-image doublets have been derived from the same strain, with a single system of nuclear apparatus. In these doublets, the dividing micronucleus is localized within one of two cortical regions similarly related to the two oral apparatuses.

K. Golińska (Warsaw) discussed the ultrastructural reorganization of *Dileptus* following excision of the proboscis. This study shows the equipotentiality of kinetosomes, through the conversion of locomotor units into sensory units with typical sensory auxiliary structures. New kinetosomes may also be seen with accessory structures different from those of the "parental" kinetosomes.

Finally S. Ferraro (Genoa, with M. G. Chessa and T. Crippa-Franceschi) described the cortical patterns manifested during division of *Colpoda cucullulus* in reproductive cysts.

Several excellent poster displays that could have been associated with these formally presented papers were also available for inspection and discussion.

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T. M. Sonneborn: Reluctant Protozoologist

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Tracy Morton Sonneborn died January 26, 1981, at the age of 75 years, leaving an indelible impression on the biological sciences in his time. The organizing committee of the VI International Congress of Protozoology has designated this session of contributed papers on genetics and morphogenesis as a tribute to Professor Sonneborn's memory. The committee has honored me by asking that I make a few remarks concerning Sonneborn's life and his contributions to protozoology.

The public record of Sonneborn's achievements is provided by his bibliography, a list of some 230 titles published over a period of half a century. Such a list documents a long, disciplined and productive career. It does not explain itself entirely, however, particularly with respect to our immediate concerns. What did Sonneborn contribute to protozoology, and what did protozoa contribute to Sonneborn's career? In order to provide a framework for a brief survey of Sonneborn's life in science, I propose a thesis that contains a mild paradox: Sonneborn's substantial scientific contributions were achieved almost exclusively through his studies of the ciliated protozoa, yet Sonneborn was reluctant to consider himself a protozoologist. As a further aid to structure, I divide Sonneborn's career into four somewhat arbitrary phases.

Phase I: The Lean Years

The first of these phases is the Hopkins phase. Sonneborn did both his undergraduate and graduate work at Johns Hopkins University

Paper presented as an introduction to the Contributed Paper Session: *Genetics and Development in memory of Professor Tracy Morton Sonneborn*. Session took place on July 7 at VI International Congress of Protozoology, Warszawa, Poland, 5–11 July 1981.

in Baltimore, Maryland, the city in which he was born. His thesis advisor, Herbert Spencer Jennings, was well-known for his experimental work on protozoa. Jennings is remembered today primarily for his definitive work on protozoan behavior; you may recall that he described the "avoiding reaction" in *Paramecium* even before the turn of the century. Yet Sonneborn did not do his thesis work on protozoa, but concerned himself instead with the inheritance of form in an invertebrate metazoan, *Stenostomum incaudatum* (Sonneborn 1930 a, 1930 b).

Sonneborn's international reputation, of course, is founded on his work with *Paramecium aurelia*, and the work on *Stenostomum* is virtually forgotten. Yet Sonneborn may have undertaken the protozoan work somewhat under duress. The "Great Depression" of the 1930's provided few opportunities for young scientists. New jobs were rare and research funds were difficult to obtain. Fortunately, Jennings' prominence as a scientist and as a spokesman for science had brought him strong and continuous research support through the Rockefeller Foundation. Because of his grant support, Jennings was able to offer a haven to Sonneborn during the lean years, provided only that he direct his efforts to the organisms specified by the Rockefeller benefactors.

I cannot know Sonneborn's feelings about these circumstances, but his postdoctoral years at Hopkins were idyllic in many ways. He had a sympathetic and supportive supervisor; he had a devoted and understanding wife; he was free from teaching responsibilities and much of the normal administrative clutter of academic life. Most importantly, he was free to investigate phenomena he found interesting, constrained only by the organismic prescription of his supporting agency. He had to work on protozoa.

Although the Hopkins years gave Sonneborn freedom, and time for his exploratory studies, they were nevertheless lean years in several ways. Sonneborn received his Ph. D. in 1928. Thereafter he worked thoughtfully and tenaciously for many years, attempting to bring *Paramecium* under experimental control. Year after year he worked with only moderate results for his efforts. Only in 1937, twelve years after beginning his graduate career, and nine years after receiving his Ph. D., did he make a really notable discovery — the discovery of "mating types" in *Paramecium aurelia* (Sonneborn 1937).

I mention the circumstances of this important event for the illumination it provides, both to Sonneborn's work and more generally to the process of discovery. Many of Sonneborn's later achievements have their origins in this early latent period. And perhaps some of the discipline and patience that harnessed Sonneborn's enormous en-

thusiasm were gained through these early days of difficulties and false starts.

In this case, fortunately, the time was available, and the tempered investigator emerged. The moment of understanding came, and with it the opportunity to open many doors. What was Sonneborn up to in these days, and why were mating types so critical for achieving his purpose? Sonneborn was above all else an experimentalist, and he was determined to bring his organism under experimental control. And by "experimental control", he meant not just growth in the laboratory, but that most subtle manipulation of biological influences — breeding analysis. Sonneborn, like Jennings, was fascinated by the enigmas of heredity. He had also grasped the strategic significance of the scientifically domesticated organism. The technological foundation for the first great surge of genetic understanding in this century was the domesticated fruit fly — *Drosophila melanogaster*. The parallel domestication of *Zea mays* provided an essential auxilliary resource.

Sonneborn and Jennings, however, perceived that genetic analysis with large complicated organisms had its limitations. They dreamed of bringing genetic studies to a deeper level of analysis using miniaturized genetic systems of simpler design, capable of being handled in larger numbers at a cheaper cost. From one perspective, Sonneborn's career can be viewed as an heroic effort to make *Paramecium* a protistan *Drosophila*. His discovery of mating types gave him the kind of control over reproductive processes necessary to that end. It gave him opportunities he exploited the rest of his life.

Phase II: *Paramecium* Goes Public

The discovery of mating types brought recognition to Sonneborn. It also brought mobility and the end of the Hopkins era. Jennings had retired at Johns Hopkins, and Sonneborn was ready to make a new start on his own. In 1939 he moved to Indiana University at Bloomington, which became his home base for the rest of his days.

The first years at Indiana, until about 1950, I will refer to as Phase II of his career. These were the days during which Sonneborn and his associates first used the tool of controlled mating to explore the basis of differences among individuals of this species. After the end of World War II, his research group grew, with many graduate students and postdoctoral fellows joining him in exciting studies.

The first organismic variations that Sonneborn subjected to breeding analysis were the mating types themselves (Sonneborn 1939). These studies were quickly followed by others on the killer characteristic (Sonneborn 1943). Still other breeding studies were car-

ried out on the inheritance of antigenic traits (Sonneborn 1948). The basic cultural methods and genetic technologies were summarized (Sonneborn 1947, 1950 a) and the first, descriptive phase of their application was essentially complete by the early 1950's. This work was meticulously documented and effectively presented to the scientific public (Sonneborn 1950 b, 1950 c). The results were unexpected and excited wide discussion. Sonneborn was himself repeatedly recognized for highly regarded discoveries.

Nevertheless, the studies also generated some dissonance in theoretical biology and genetics. The overwhelming conclusion of classical genetic studies was that hereditary differences between individuals are controlled by chromosomal differences. Much of the work on *Paramecium*, though providing abundant evidence of Mendelian control of organismic capabilities, revealed complicated relationships between the genes, the cytoplasm and the environment in the expression and perpetuation of cellular differences. In particular, although each of the systems studied in *Paramecium* is different, they all manifest in some circumstances the phenomenon of "cytoplasmic inheritance" or "uniparental transmission".

The *Paramecium* work challenged geneticists to a more comprehensive understanding of the functional interactions of the components of the cell. Unfortunately, the time for that challenge was not ripe, and it has not yet been fully met. To understand what happened to *Paramecium* genetics, we need to take a broader view of what was happening in biology. The dream of a microbial genetics was not the sole property of Jennings and Sonneborn. Beadle and Tatum, and Luria and Delbruck also undertook the domestication of microbes as scientific instruments. And they chose organisms even smaller and simpler than *Paramecium*, organisms more congenial to the biochemical technologies available. Using these organismic tools, the genetic community developed a driving preoccupation to dissect the nuclear apparatus. *Escherichia coli* and its phages became the workhorses of genetics. The complicated and confusing ciliates and the problems they raised were set aside for later consideration.

Phase III: *Paramecium* as an Organism

I think that Sonneborn recognized something of these complex sociological events of the 1950's. He joined fully in the excitement of discoveries concerning the structure of DNA, the synthesis of proteins, the regulation of primary gene activity. But even though his area was by-passed in a sense, he did not abandon his efforts to domesticate *Paramecium* more completely or to use it in sophisticated analyses.

Moreover, he must have been caused to wonder why the ciliates gave such unexpected answers to simple questions. In any case, for the next few years, until about 1960, Sonneborn gave a lot of thought to the ciliates as real organisms in Darwinian time and space. This period I refer to as Phase III, and its most characteristic expression was his long review in 1957 on the species problem in protozoa (Sonneborn 1957).

This period is the one most productive of what one is inclined to designate as "protozoological" contributions, as opposed to "genetic" contributions. And perhaps this is as good a place as any to recognize Sonneborn's ambivalence toward "protozoology". Sonneborn did not work on protozoa for his doctoral thesis, and he may have begun to work with *Paramecium* at least in part through economic necessity. He taught protozoan genetics, but only as a periodic subject, alternating it with bacterial genetics, fungal genetics, and algal genetics. The one time he tried to teach protozoology, he became fascinated with a sample of pond water and managed to spend the whole semester working with the class on a few particular species. In the 1940's, he was opposed to the formation of a Society of Protozoologists, and in the 1950's he thought a journal devoted exclusively to the protozoa was an unfortunate conception. He never held high office in the Society of Protozoologists, though his *curriculum vitae* is loaded with services to other scientific societies.

I suspect that Sonneborn's attitude toward protozoology was connected with his hope that *Paramecium* would be recognized as a generalized cell and not as a ciliate, just as *Drosophila* was recognized in some sense as a generalized organism and not as an insect. He did not want his organism to be limited by its association with some peculiar, atypical, aberrant group. He did not want his discoveries published in a parochial journal read only by a partisan readership. Moreover, he refused to limit his own interests according to taxonomic criteria. He wasn't interested in *Paramecium* because it was a protozoan, but because it was alive.

The fundamental issue here probably lies in the diversity of the commitments of biologists. Some biologists become fixed on a particular phenomenon or structure, and pursue it through a multiplicity of organisms and with a variety of techniques. Others master a methodology and adhere to it while varying the objects to which it is applied. And, of course, some biologists make a primary organismic investment. And organismic biologists differ, not only with respect to the groups that attract their attention, but also with respect to the sizes of the groups. Some biologists direct concentrated attention to one species or to a small group of species; others give more diffuse attention to a much larger group. I am not entirely sure how a "protozoologist" is defined,

but the term almost certainly implies a special interest in all the organisms classified as protozoa, and also a diminished interest in the organisms excluded from the category. If so, Sonneborn was not a protozoologist. He was never interested in any organism simply because it was a protozoan, and he refused to have his interests in other organisms restricted. I suspect he would have been amused by the confusion and diversity of opinion manifested in these meetings concerning what, if anything, a protozoan is. The debate would not have affected his identity. He was unequivocally a biologist, a geneticist, and a parameciologist.

I suspect that his achievement of the status of parameciologist came belatedly. I remember being startled by his opinion that only specialized mechanisms are truly interesting. This opinion of the 1960's would have been intolerable in 1950, and it indicated a revision of values. I believe that Sonneborn was converted when he accepted the challenge to explain the discrepancy between the studies on *Paramecium* and those on fungi and bacteria. Why should ciliates give such different results in breeding studies? What kind of an organisms is a *Paramecium* anyway? The search for the universal principles would have to wait for a deeper understanding of the particular.

During the 1950's Sonneborn set about assembling, in his systematic way, all the information available on the biological characteristics of the beasts that circumstances had brought to be his companions. He wanted to know about their life histories, their distributions, their mating habits, their evolutionary relationships. The observations go back to the time that mating types were discovered, but their use in systematic comparative study was new.

I cannot adequately summarize here the masterly multi-dimensional synthesis of paramecium biology that Sonneborn produced at this time, but I must recall a few isolated elements. First, let me mention the phenomenon of cryptic species. The strains of *Paramecium aurelia* do not constitute a single large gene pool, but are broken up into many completely isolated non-interbreeding Mendelian populations. Using the techniques Sonneborn developed, other workers have shown that many "named" species of ciliates have the same kind of cryptic genetic complexity: genetically isolated populations of remarkably similar phenotypes, yet of great diversity of genetic structure and evolutionary history. This observation has not endeared Sonneborn to systematic protozoologists, or to ecologists forced to deal as best they can with manifest variation instead of cryptic genetic differences. Sonneborn's work strongly supports the biases of the "splitters" in their conflicts with the "lumpers", at least for many species of ciliated protozoa. The cryptic species of ciliates, on the other hand, are an unexpected

gift to comparative biochemists, and a powerful instrument for studying the evolution of cellular structures.

A second protozoological insight came from asking about how the persistent cryptic species maintain their places in nature over extravagantly long periods of time. Their large phenotypic and distributional overlap leads one to expect some subtle microecological distinctions. In fact, Sonneborn found the clue to their distinctiveness in what may be referred to as their "ecogenetic strategies". At the simplest level, sibling species are distinguished by different positions on an inbreeding-outbreeding scale. Some species are inbreeders, committed to mutational variety as a way to meet environmental challenges; others are outbreeders and rely importantly on recombinational variety. These generalizations are imbedded in a rich tapestry of relationships involving many facets of the life history, the aging process (Sonneborn 1954), the mating system.

This synthesis preceded the recognition and study of ecogenetic systems in other organisms by many years. Sonneborn's work was not fully appreciated, however, either by protozoologists or by population biologists who should have been aware of what he was attempting. I am pleased to see the modern resurgence of ciliate work in this mode, particularly by the Italian school.

Phase IV: *Paramecium* as an Instrument

The fourth stage of Sonneborn's career I am inclined to view as his mature phase. By 1960, Sonneborn had a firm grasp of the experimental capabilities of *P. aurelia*, and also of its limitations. He had faced and accepted its peculiarities, and could choose for experimental analysis phenomena he considered interesting and important, with a realistic understanding of what could and could not be achieved.

It was during this time that I first became fully aware of his policy not to do an experiment that someone else was likely to do. Certainly it was implicit in his earlier practices, particularly in turning over to students and associates major projects that he had initiated. But I think that his mature phase was characterized by a fuller appreciation of the multitude of natural phenomena begging for analysis, and with the limited number of workers available to carry out those analyses. More than ever he encouraged students and colleagues to do their own things, to respond directly to their own observations and to determine their own priorities.

He was always marvelously perceptive of deep issues in trivial manifestations. I remember being surprised by his determination to

study the hereditary basis of doublet paramecia. He asked me what I thought would happen if he crossed a singlet with a doublet. I replied that certainly one exconjugant would remain a singlet and the other would remain a doublet. He agreed, but he also asserted that therein resides a mystery for which conventional genetic wisdom has no explanation. And he announced that he would do that experiment with such sophisticated controls that no one could ever again doubt that the continuity of form in cellular lineages involved some new and important principles (Sonneborn 1963, Beisson and Sonneborn 1965).

I must admit to thinking in 1960 that Sonneborn was not entering his mature phase, but rather passing beyond it into his senile phase. Nothing seemed duller to me at that point that cortical morphogenesis. Yet within a few years Sonneborn's doublet work had recruited a new array of talented experimentalists into ciliate work. And even I, who had earlier refused to look at a silver — stained specimen, found myself caught up in the excitement of "cytotaxis", "structural guidance", and "positional information". Sonneborn's demonstration of the role of cellular fabrics in directing the assembly of organelles was a major contribution to biological thought, with applications far beyond the limits of the ciliates (Sonneborn 1967, 1970 a).

Leaving *Paramecium* morphogenesis in good hands, Sonneborn took up as his last major experimental project, the understanding of yet another apparently trivial phenomenon — the failure of certain long-maintained strains to express an hereditary characteristic they had once manifested. This difference concerned the ability of the cells to discharge their trichocysts. In a characteristically elegant study, Sonneborn demonstrated that these hereditary differences arise as consequences of developmental events in growing new macronuclei (Sonneborn and Schneller 1979). He connected this phenomenon with the earlier studies on mating type determination, and pointed to editorial alterations of the macronuclear chromatin as the basic mechanism. This work brought Sonneborn back into convergence with a major stream of inquiry. Similar phenomena are now being recognized in many other organisms, in mice and maize and *Drosophila*, in yeast and *Salmonella*. The difference is that the phenomena once considered special and confusing, are now considered general and capable of resolution (1977).

After a recent conference on experimental ciliatology, Sonneborn remarked that he felt a little like Moses must have felt, privileged to look into the Promised Land, but not allowed to go in himself. The more complex systemic properties of cells, and the interactive roles of cell components in cellular heredity, are at last receiving attention;

powerful new technologies are resolving mysteries that Sonneborn helped define. The ciliated protozoa will play a role in future advances primarily because T. M. Sonneborn prepared them for such a role (Sonneborn 1970 b). Not just protozoology, but all biology is indebted to him.

Conclusion

One cannot in half an hour do justice to half a hundred years of distinguished science. I have tried to focus attention on Sonneborn's scientific, and particularly protozoological achievements, but these can scarcely be considered in isolation from his personal characteristics. Sonneborn's personal qualities attracted to experimental ciliatology an international community of superlative investigators, who have in concert achieved far more than he alone could have accomplished.

I recently asked a friend of mine who is a distinguished historian of science, why the concept of "catastrophism" went into eclipse in the early 19th century. It was an exciting idea; it was supported by the simplest reading of the record in the rocks and by the mythologies of many cultures; it was promoted by the brilliant and charismatic Cuvier. Yet the dull but sophisticated concept of "gradualism" won out for a time in favor over catastrophism. A century and a half later were are returning to a serious consideration of saltatory and catastrophic events in the history of life. Why were Cuvier and his ideas passed over? The answer, according to Burkhardt, probably lies in recruitment. Cuvier was unable to attract and sustain vigorous students and colleagues. The uniformitarians, on the other hand, were not dominated by an inhibitory genius, but consisted of a diversified collection of independent and mutually supporting scientists.

Perhaps Sonneborn's most lasting contribution to protozoology has been his recruitment to the protozoa of an exceptional crop of tough-minded, if warm-hearted, experimentalists. He contributed to these recruits a commitment to rigor coupled with an excitement concerning both the organismic particuliarities and their general extrapolations. Moreover, he established a tradition of mutual respect and cooperation among the workers that is distinctive in a time of personal, professional and national insecurity. The sense of community among ciliate experimentalists is a rare and precious legacy.

Those of you who knew Tracy Sonneborn realize that the fostering of a sense of community was not Tracy's work alone. He was aided and abetted every step of the way by his companion through the years. When Ruth Sonne-

born heard that this session was to be dedicated as a memorial to Tracy, she asked if she could send a brief message, which I hereby transmit:

June 26, 1981

Dear Friends of Tracy and of mine

Your letters to him while he was ill and to me during these past weeks made us realize how much you are part of our lives, and the scientific quest which you shared with him; as well as the affection and concern which you have given. I hope soon to write to you individually and I do read and reread your messages. I am busy trying to get Tracy's files ready for the library so that some of his life may be available if any of you want reprints or manuscripts. Many of you are represented with correspondence and your own reprints among these papers.

What fortunate people we have been and what a fine spirit of "give and take" has existed among us, from so many different countries, so many different undertakings. Tracy ended his Camerino remarks:

"I think that the future holds promise for ciliate work at every level-from populations to cells, and on down — but the generation of opportunities and advances, especially the unpredictable ones, depends also on individuals who follow their own curiosities and interests. Good Hunting and Much Joy in the Search".

I hope that this meeting brings you new understandings of your own work and that of your colleagues, and some new vistas.

My gratitude and affection,

Ruth Sonneborn

REFERENCES

Beisson J. and Sonneborn T. M. 1965: Cytoplasmic inheritance of the organization of the cell cortex in *Paramecium aurelia*. Proc. Nat'l. Acad. Sci. USA, 53, 275–282.

Sonneborn T. M. 1930 a: Genetic studies on *Stenostomum incaudatum* (nov. spec.). I. The nature and origin of differences among individuals formed during vegetative reproduction. J. Exp. Zool., 57, 57–108.

Sonneborn T. M. 1930 b: Genetic studies on *Stenostomum incaudatum*. II. The effects of lead acetate on the hereditary constitution. J. Exp. Zool., 57, 409–439.

Sonneborn T. M. 1937: Sex, sex inheritance and sex determination in *Paramecium aurelia*. Proc. Nat'l. Acad. Sci. USA, 23, 378–385.

Sonneborn T. M. 1939: *P. aurelia*: mating types and groups; lethal interactions: determination and inheritance. Am. Naturalist., 73, 390–413.

Sonneborn T. M. 1943: Gene and cytoplasm. I. The determination and inheritance of the killer character in variety 4 of *P. aurelia*. II. The bearing of determination and inheritance of characters in *P. aurelia* on problems of cytoplasmic inheritance, pneumococcus transformations, mutations and development. Proc. Nat'l. Acad. Sci., USA, 29, 329–343.

Sonneborn T. M. 1947: Recent advances in the genetics of *Paramecium* and *Euplotes*. Adv. Genet., 1, 263–358.

Sonneborn T. M. 1948: The determination of hereditary antigenic differences in genically identified *Paramecium* cells. Proc. Nat'l, Acad. Sci., USA, 34, 413–418.

Sonneborn T. M. 1950 a: Methods in the general biology and genetics of *P. aurelia*. J. Exp. Zool., 113, 87–143.
Sonneborn T. M. 1950 b: The cytoplasm in heredity. Heredity 4, 11–36.
Sonneborn T. M. 1950 c: Partner of the genes. Sci. Amer. 183, 30–39.
Sonneborn T. M. 1954: The relation of autogamy to senescence and rejuvenescence in *Paramecium aurelia*. J. Protozool., 1, 38–53.
Sonneborn T. M. 1957: Breeding systems, reproductive methods and species problems in *Protozoa*. In: The Species Problem (ed. E. Mayr), 155–324. Amer. Assoc. Adv. Sci., Washington, D. C.
Sonneborn T. M. 1963: Does preformed cell structure play an essential role in cell heredity? In: The Nature of Biological Diversity, (ed. J. M. Allen) 165–221, McGraw Hill, New York.
Sonneborn T. M. 1967: The evolutionary integration of the genetic material into genetic systems. In: Heritage from Mendel (ed. R. A. Brink), 375–401. University of Wisconsin, Madison and London.
Sonneborn T. M. 1970 a: Gene action in development. Proc. Roy. Soc. Lond. B., 176, 347–366.
Sonneborn T. M. 1970 b: Methods in *Paramecium* research. Methods of Cell Physiol., 4, 241–339.
Sonneborn T. M. 1977: Genetics of cellular differentiation: stable nuclear differentiation in eukaryotic unicells. Ann. Rev. Genetics, 11, 349–367.
Sonneborn T. M. and Schneller M. V. 1979: A genetic system for alternative stable characteristics in genomically identified homozygous clones. Devel. Genetics, 1, 21–46.

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