PL ISSN 0065 - 1583

POLISH ACADEMY OF SCIENCES
NENCKI INSTITUTE OF EXPERIMENTAL BIOLOGY

ACTA PROTOZOOLOGICA

VOLUME 22

Number 1

W A R S Z A W A 1 9 8 3

POLISH ACADEMY OF SCIENCES
NENCKI INSTITUTE OF EXPERIMENTAL BIOLOGY

ACTA PROTOZOOLOGICA

International Journal of Protozoology

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ACTA PROTOZOOLOGICA appears quartely. The indexes of volume 21 will appear in No. 2 of volume 22.

Indexed in Current Contents and in Protozoological Abstracts.

ACTA PROTOZOOLOGICA
Vol. 22, No. 1, pp. 1–18 (1983)

Contraction and Streaming Relations Recorded Simultaneously at Two Points Along the Plasmodial Veins and Frontal Channels of *Physarum polycephalum*

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Received on 10 September 1982

Synopsis. The contraction : relaxation time ratio amounts to 48 : 52 and the forward : backward streaming time ratio to 55.5 : 44.5, the proportions varying insignificantly between different control sites. Contractions and streamings are both positively but weakly correlated between the sites situated in the veins region. Forward streamings in veins are positively correlated to local contractions and to contractions taking place behind the control site. The correlation between the forward streamings and local contractions which is positive in veins, becomes negative in frontal channels. The forward streaming in frontal channels is equally well correlated with their own relaxation as with the contraction of veins lying behind them. The time difference between the duration of forward and backward flow periods in the veins is established mainly during their contraction, but in frontal channels during their relaxation.

The quantitative studies of time and space relations between the phases of contraction–relaxation and forward-backward streaming oscillations are of great interest for understanding the coordination and integration of the motile activities in slime moulds plasmodia.

The first attempt to demonstrate the positive correlation between the forward streaming periods and the local contraction phases was made, in medium size branching veins of the plasmodial network, by Grębecki and Moczoń (1978). Later on, we examined the changes of the same parameter, and some others, measured separately in different experiments at various locations across the network of veins (Kołodziejczyk and Grębecki 1982). Finally, we followed the differences in the contraction : streaming relations separately in

the network veins and in the frontal channels, after exposing one of these regions to the white-red illumination changes (Kołodziejczyk and Grębecki 1983).

A serious limitation of conclusions drawn from two our earlier studies resulted from the fact that in each experiment the contractile activity and the flow direction were controlled only in one spot at once. So for example, the differences in the contraction : streaming relations between two distant places had to be evaluated on the basis of mean results produced by two separate series of experiments. The streaming oscillations could be correlated to the local contractile activity, but not to contractions occurring anywhere else. Therefore, it became the objective of the present study to re-examine the space distribution of some contraction and streaming parameters across a plasmodium, with such a method which would permit to keep both phenomena under simultaneous control at two different localizations. The coupled pairs of records produced in that way will enable us to confront the behaviour of two different regions of a plasmodium directly, i.e., in the same individual, under the same conditions and at the same time.

Material and Methods

The culture technique and the methods of manipulating plasmodia before experiments were the same as described earlier (Kołodziejczyk and Grębecki 1982).

The scheme of the experimental set is shown in Fig. 1, and the consequent steps of enlarging and splitting the image of plasmodium in Fig. 2. An incandescent lamp with heat filter served as the source of light (SL). A Fresnel lens (Fr) was interposed between the light source and the subject. It ensured a perfectly uniform white illumination of 8000 lux at the plane of observation. The illuminated field was considerably larger than the maximal size of examined plasmodia. The light intensity was certainly very high, when compared to that used in our earlier experiments, but at lower illumination levels the quality of the final image was insufficient to survey the streaming oscillations.

Plasmodia (Pl) migrating on the agar gel were kept in the inverted Petri dishes. A Holos wide angle microscope lens (L_1) was focused upon the selected segment of a vein, about 15 mm long, and formed its primary image (I_1) 10 times magnified. Two separate TV cameras (TVC), equipped with 5× microscope lenses (L_2) mounted on the extension tubes, were focused on two points situated 120 mm (= 12 mm natural size) apart one from another, along the enlarged image of the vein's segment. Their secondary images (I_2) formed at the photosensitive surface of the cameras were transmitted to two monitors (TVM). Their final images produced on the screens (TVI) were eventually magnified 380 times.

Two selenium photoelements (PhE) controlled through photographic lenses (L_3) two selected spots, one on each screen. The changing diameter of pulsating veins produced oscillations in brightness of the surveyed fields on the screens, and the

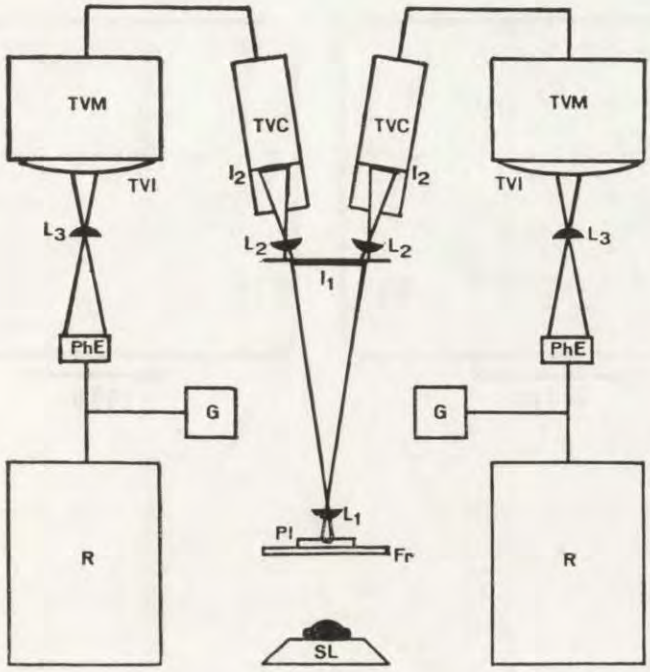

Fig. 1. Block scheme of the experimental equipment set. SL — source of light, Fr — Fresnel lens, Pl — plasmodium in Petri dish, L_1 — Holos wide angle lens, I_1 — primary image of the selected vein's segment, L_2 — two 5× microscope lenses, I_2 — secondary images of two extremities of the vein's segment, TVC — television cameras, TVM — television monitors, TVI — final images produced on the TV screens, L_3 — photographic lenses, PhE — photoelements, R — chart recorders, G — low frequency generators

corresponding photocurrent oscillations were transmitted to the recorders (R). The plotted curves were considered as reflecting the contraction–relaxation periodicity of the examined areas. The changes of the streaming direction in them were visually surveyed on the TV screens and the information transmitted to the same recorders by operating the auxiliary low frequency generators (G).

The observations were run in 36 pairs of control sites located along the veins and in 18 pairs in which the veins were compared to the frontal channels.[1] The control sites within a pair were always selected at the distance of 12 mm from one another. In the first group of records 20 veins produced secondary ramifications between the control sites and 16 were not branched, but the differences

[1] The term: frontal channels is applied by the authors to the small streamlets of flowing protoplasm, which appear just behind the advancing frontal ridge of plasmodium. They are running across the continuous protoplasmic layer, in which the veins are not yet individualized. The term: veins is consequently reserved for the streams bounded within the differentiated tube walls, separated one from another in the form of an open network, which is typical in the "older" parts of a migrating plasmodium.

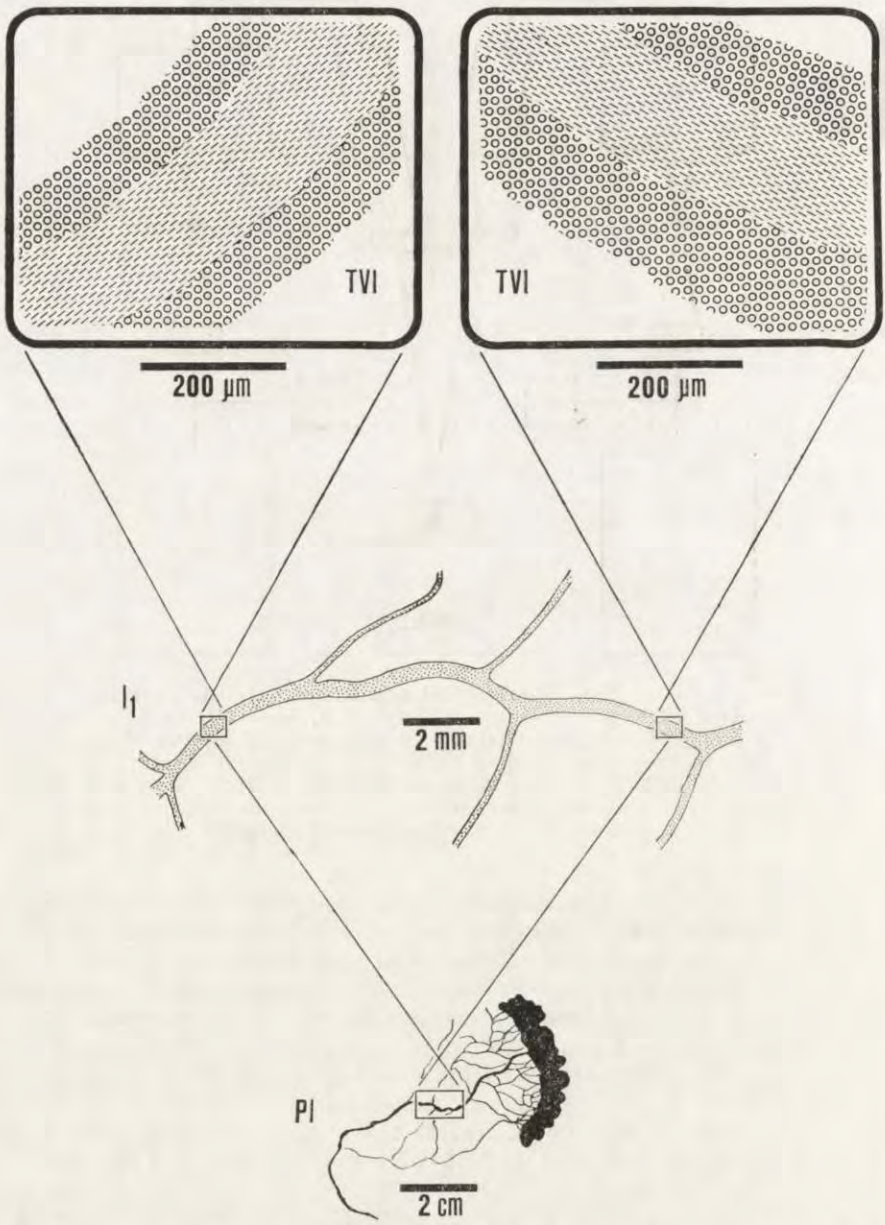

Fig. 1. The drawing of the whole plasmodium (Pl) and the primary image of the segment by the equipment shown in Fig. 1. The same lettering is applied as in Fig. 1. The drawing of the whole plasmodium (Pl) and the primary image of the vein's segment (I_1) are reduced to about 1/2, and the final images appearing on the TV screens (TVI) to about 1/4 of their natural sizes

in their behaviour proved not to be conclusive and consequently they were treated together as one experimental series.

Each individual pair of records was taken for 1000 s. The data were read out from the curves at the points corresponding to 5 s time intervals, i.e., 200 scores were collected per experiment. Therefore, the correlation coefficient $\varphi = \sqrt{\chi^2/N}$ for each individual was significant at $p = 0.01$, when it exceeded ± 0.18. But the mean φ values were calculated directly from the sum of basic data, which produced the data populations of 7200 for the veins and 3600 for the veins-front coupled records. For the whole groups of experiments therefore, the correlation between any two investigated parameters was significant at the 0.01 level when $\varphi > \pm 0.03$ or $\varphi > \pm 0.05$, respectively.

Results and Discussion

Contraction : Contraction and Streaming : Streaming Correlation Between Two Distant Sites

In average, the contraction phases are positively correlated in time between two points selected in the middle course of a major vein and spaced at 12 mm from one another. The mean value of the correlation coefficient φ amounted to 0.15 and 72% of the investigated plasmodia showed a positive significant correlation (Table 1).

It can be stated that the majority of the examined group behaved in the manner confirming the conclusion of Grębecki and Cieślawska (1978) that plasmodium "represents an imperfectly synchronized monorhythmic contractile system". It should be stressed on the other hand, that not all the specimens behaved in the expected manner and that the mean φ value was rather low. Certainly, it may be attributed to the discoordinating effect of the white light which intensity in the present experiments amounted to 8000 lux (but only 80 lux in those of Grębecki and Cieślawska 1978). It is known that an intense white (or blue) light desynchronizes contractions in the interconnected isolated veins (Takeuchi and Yoneda 1977) and produces pronounced irregularities in the oscillatory phenomena surveyed in the double chamber (Hato et al. 1976, Rakoczy unpublished), tensiometrically (Hato et al. 1976, Block and Wohlfarth-Bottermann 1981), or photometrically *in situ* (Kołodziejczyk and Grębecki 1983).

The data presented in the Table 1 demonstrate that the protoplasmic streaming directions are, in most cases, also positively correlated between two control sites situated along a major vein of the network. As well the correlation coefficient ($\varphi = 0.25$) as the number of positive correlation cases (82%) were even higher for the streaming oscillations than for the contraction–relaxation cycles. Grębecki and Moczoń (1978) stated that the synchronization of streamings is weaker than

Table 1

Correlation of contraction and streaming oscillations between two distant sites

Sites under control	Correlated phenomena	Mean		Relative frequency of the cases of:		
		number of plasmodia	correlation coefficient φ	positive correlation	insignificant	negative correlation
Both along a major network vein	contractile activity	36	0.15	72	12	16
	streaming direction	36	0.25	82	12	6
One in a vein, another in frontal channels	contractile activity	18	0	40	33	27
	streaming direction	18	0.01	33	28	39

that of contractions, but this conclusion concerned the relations between two branching veins, and not between two points along the same vein.

When the contraction or streaming oscillations are compared between one site selected on a vein of the network and the second situated inside the frontal zone, where the protoplasm flows in small streamlets, the situation becomes different. The results summarized in the Table 1 show an apparent lack of any relation between the oscillations recorded simultaneously in the veins and in the frontal channnels.

The frontal channels kept under control in this experiment were situated between the network of veins and the frontal edge in which the contractio nand relaxation phases alternate (Grębecki and Cieślawska 1978, Kołodziejczyk and Grębecki 1980). Therefore, the results shown in the Table 1 may be interpreted not as a manifestation of independent behaviour of the investigated parts of plasmodium, but as an intermediary state of transition from the positive correlation (veins *vs.* veins) to the negative correlation (veins *vs.* frontal edge). It should be added that the frontal channels lie on the territory where the phase shifts and the resulting wave phenomena were reported by several authors (e.g., Baranowski 1976 b, 1978).

Random Character of the Non-synchronous Behaviour

The important proportion of cases when the both ends of the investigated segment of a vein differ in phase of their contractile activity or flow oscillation leads us to examine the possible bearing of such situations on the mechanism of movement.

One might suppose for example, that not the synchronous but the non-synchronous contractions are responsible for the directional flow of protoplasm. It would mean in other words that the streaming flows always and anywhere from a contracting site to a relaxing one. The inadequacy of such a simplified view has been theoretically discussed by Jahn (1964). The present results revealed that in fact the streaming directions are not strictly obeying the local contraction-relaxation differences. And *vice versa*, their distribution does not become random when the both ends of the investigated segment contract or relax in unison. It seems that the major polar differences across the whole plasmodium (probably the veins-front polarity) easily overhealm the minor local oppositions in phase of the contractile activity.

Theoretically, there is also a possibility that the deviations from

Table 2

Forward and backward propagation of contractions and of streaming reversals

Sites under control	Investigated phenomena	Number of plasmodia	Number of cycles	Relative frequency of changes beginning:		
				from the posterior end	synchronously[1]	from the anterior end
Both along a major network vein	contractile activity	23	251	47	10	43
	streaming direction	23	251	44	13	43
One in a vein, another in frontal channels	contractile activity	16	193	47	9	44
	streaming direction	16	193	44	7	49

[1] According to the present methods the time differences < 5 s had to be considered as null.

the synchronous oscillations pattern, as described in the precedent paragraph, are due to some regular shifts of phase between the two investigated sites. It is conceivable, for example, that contractions and/or streaming reversals are consequently propagated across the network of veins from behind toward the frontal areas or in the opposite direction. Such a possibility has been envisaged by some earlier authors (Seifriz 1953, Stewart 1964, Rhea 1966, Durham and Ridgway 1976).

The hypothesis of directionally propagated contraction and streaming reversal waves was checked in 23 pair of cures plotted along the major network veins, which were enough regular to allow the identification of the same phases in both coupled records. In that way, in total 251 contraction-relaxation cycles, and the same number of the forward–backward streaming cycles, were studied. The results shown in the Table 2 prove that in the network area the contractions and the forward streaming may begin indifferently, as well from the posterior as from the anterior end of the controlled segment of vein. The analysis of 16 pairs of curves recorded simultaneously in the veins and in frontal channels resulted also in an almost random data distribution.

The obtained results confirm the impression made by the ciné-film produced by Cieślawska and Grębecki (1979), that the deviations from the synchronous contraction pattern in the veins are chaotic and they do not represent, therefore, any different mode of coordinating the motile phenomena in plasmodium.

Since the direction changes of the protoplasmic flow at the two control sites are seldom exactly synchronous, the streaming reversal involves in most cases a temporary coexistence of two opposite streamings in the investigated segment of a vein. They may be either convergent or divergent, depending from which side the streaming reversal or re-reversal is beginning. The possible role of such "afferent" and "efferent" flows in the mechanism of movement of plasmodium has been in the past strongly emphasized by Stewart (1964). In the same experimental material which was used in the Table 2, both situations were encountered with the same frequency (243 cases of convergent streamings and 246 of the divergent flow). The convergent streamings in 53% resulted in the establishment of uniform backward flow, whereas the divergent ones finished by the "choice" of the backward direction in 46% of cases. This difference appears too weak to be considered as conclusive. So, the relevance of the "afferent" and "efferent" flow episodes for the mechanism of movement seems to be doubtful.

Table 3

Correlation of the forward streaming phases with contractions taking place at the same control site

Sites under control	Streaming and contractions recorded in:	Number of plasmodia	Mean correlation coefficient φ	Relative frequency of the cases of:		
				positive correlation	insignificant	negative correlation
Both along a major network vein	the posterior control site	36	0.20	76	8	16
	the anterior control site	36	0.11	61	13	26
One in a vein, another in frontal channels	a vein behind the front	18	0.26	83	11	6
	frontal channels	18	−0.09	27	17	56

Correlation of Forward Streaming with Local Contractions

During the regular progressive motion of a fairly well synchronized plasmodium the protoplasm should be squeezed from the veins forward, toward the frontal zone, mainly at the veins' contraction phase (Grębecki and Cieślawska 1978). A weak correlation in time between the contraction of veins and the forward streaming was found by Grębecki and Moczoń (1978), and a much stronger by us (Kołodziejczyk and Grębecki 1982). The present data, which seem to occupy the intermediary position between these two earlier estimations are presented in Table 3. In the Table 5 A they are compared to the results reported in other papers.

The attention should be drawn to the fact that the variations in the correlation coefficient φ values obtained in all these studies seem to be in a great extent dependent on the light conditions, during and before the experiment. The uniform illumination of 8000 lux, used in the present experiments, was certainly more appropriate than $>$ 12 000 lux concentrated in the limits of the field of view, but less favourable than 700–1400 lux of the white light or 350–700 lux of the red light. It explains why the φ values established in the present study are higher than those found by Grębecki and Moczoń (1978), but lower than reported by us elsewhere (Kołodziejczyk and Grębecki 1983). It may be concluded that the correlation between the forward streaming and contractions is strongly affected by the white light which discoordinates these both phenomena.

The present results (Table 3) demonstrate that, when the contraction : streaming correlation is measured simultaneously at two sites along a principal network vein, it is usually revealed stronger at the posterior end of the investigated segment. It confirms our earlier observation that the correlation measured separately at three different locations scattered across the network decreases in the forward direction (Kołodziejczyk and Grębecki 1982). When one of the two control sites is situated in the frontal channels zone (Table 3), negative values of the coefficient φ are there obtained. Similar negative φ values in that frontal area are reported by us elsewhere (Kołodziejczyk and Grębecki 1983).

It may be generalized therefore, that the forward streaming direction depends on the veins contraction in the posterior and central parts of plasmodium, this relation weakens on approach to the frontal zone, and in the frontal channels on the contrary, the forward direction of the protoplasmic flow depends on channels' relaxation.

This conclusion emphasizes again the functional opposition of the network of veins and of the front during the locomotion of plasmodium.

Correlation of Forward Streaming with Distant Contractions

The technique of monitoring the contractile activity and protoplasmic flow oscillations simultaneously at two distant control sites allowed us to study also the dependence of the forward streaming on contractions observed elsewhere, behind or in front of the site at which the flow direction was recorded. The results are shown in the Table 4.

Table 4

Correlation of the forward streaming phases with distant and local contractions (φ)

Sites under control	Forward streaming recorded in:	Correlated to contractions recorded:		
		12 mm behind	at the same place	12 mm toward the front
Both along a major network vein	the posterior control site	—	0.20	−0.03
	the anterior control site	0.15	0.11	—
One in a vein, another in frontal channels	a vein behind the front	—	0.26	−0.01
	frontal channels	0.10	−0.09	—

The correlation coefficients between the streamings and contractions fall to insignificant values close to 0, if contractions are not recorded at the same place as the streamings, but 12 mm away in frontal direction. But the forward streamings prove, on the contrary, to be in each case clearly dependent on contractions taking place behind them. Along the examined segments of major network veins, the forward flow in their anterior extremities is slightly better correlated with the contractile activity of their posterior, ends than with their own local contractions. This difference becomes much more pronounced in the case of streamlets crossing the frontal zone. The forward streaming direction in that area is in the same degree correlated with the contraction phase of the veins lying behind ($\varphi = + 0.10$) as with the relaxation phase of the frontal channels themselves ($\varphi = - 0.09$). These results fit in very well with all the concepts which recognize that streaming in a plasmodium follows the differences of hydrostatic pressure, that the contractile activity of veins is the main source of the motive force, and that the contractions in the veins and in the front are antagonized.

Contraction : Relaxation and Forward : Backward Streaming Time Ratios

The ratios between the duration time of contraction and relaxation phases were calculated for each one of the four sites kept under control in the two groups of experiments. In the 12 mm long segments of the major network veins the mean contraction : relaxation time ratio amounted to 45 : 55 at the posterior ends and 47 : 53 at the anterior ends of the investigated segments. The proportion 49 : 51 was obtained in the frontal channels, and 46 : 54 in the veins lying behind them. These data, together with very similar results reported in other papers (summarized in the Table 5 B), lead to the conclusion that the contraction times are very steady. They were not significantly influenced neither by the type and localization of the investigated channel nor by the illumination differences appearing in the quoted studies. The concurrence of all the data collected in the Table 5 B allows the general estimation that the contraction phases are slightly but invariably shorter than the relaxation phases and they cover in average 48% of the total observation time.

It would be prematurate to speculate about the meaning of this fact. Moreover, it should be kept in mind that the word contraction is used here in its phenomenological sense, because the contraction and relaxation phases were in the reality revealed as oscillations in diameter of the examined veins or channels.

Also the total forward : backward streaming time ratio (Table 5 C) proved not to be a parameter very sensitive to the position of a vein in plasmodium or to its illumination. The mean ratios established in present experiments ranged from 55 : 45 to 57 : 43 between the posterior and anterior ends of the investigated segments of network veins, amounted to 54 : 46 in the frontal channels and to 57 : 43 in the veins situated behind them. Our earlier observations made under different conditions (Kołodziejczyk and Grębecki 1983) gave very similar results. Although Grębecki and Moczoń (1978) obtained a lower ratio, and Anderson (1951) estimated that both streamings are of equal duration, we consider now legitimate the conclusion that in average the protoplasm is flowing forward during 55.5% of the total time of the shuttle streaming oscillations.

It means, in other words, that the forward streaming phases are in average 25% longer than the periods of the reverse flow. This difference seems to be so regular and enough important to explain the net gain of the protoplasm volume transported eventually in the frontal direction. Of course, the importance of the time factor in

Table 5

Review of data concerning the contraction and streaming relationships collected in the present paper and in some earlier studies

Type of veins and localization	Illumination	(A) Contraction: forward streaming correlation (φ)	(B) Contraction: relaxation time ratio	Forward: backward streaming time ratio			References
				(C) Total	(D) During contraction	(E) During relaxation	
Medium size veins of the network	>12000 lux, localized white light limited to the field of view	0.16	48 : 52	52 : 48	57 : 43[1]	47 : 53[1]	Grębecki and Moczoń (1978)
Major network veins: (a) posterior segment (b) middle course (c) close to the front	700–1400 lux, uniform white light (preadapted in darkness)	0.42 0.35 0.13	48 : 52	55 : 45	72 : 28[1]	39 : 61[1]	Kołodziejczyk and Grębecki (1982)
Major network veins	700–1400 lux, uniform white light (preadapted in white light)	0.16	47 : 53[1]	55 : 45[1]	63 : 37	45 : 55	Kołodziejczyk and Grębecki (1983)
	350–700 lux, uniform red light (preadapted in red light)	0.36	48 : 52[1]	56 : 44[1]	74 : 26	39 : 61	
Two points along a major network vein	8000 lux, uniform white light	0.20–0.11	45 : 55–47 : 53	55 : 45–57 : 43	66 : 34–62 : 38	46 : 54–51 : 49	This paper
Frontal channels	700–1400 lux, uniform, white light (preadapted in white light)	−0.09	49 : 51[1]	56 : 44[1]	52 : 48	61 : 39	Kołodziejczyk and Grębecki (1983)
	350–700 lux, uniform red light (preadapted in red light)	−0.12	48 : 52[1]	56 : 44[1]	49 : 51	62 : 38	
Frontal channels	8000 lux, uniform white light	−0.09	49 : 51	54 : 46	49 : 51	58 : 42	This paper

[1] Data not appearing in that form in the papers quoted, but calculated again from the original documentation

polarizing the protoplasm net transport does not exclude the possible contribution of the velocity and vein's diameter factors, as stressed by Baranowski (1976 a).

Role of Contraction and Relaxation in Polarizing the Protoplasm Transport

If the total contraction time is always slightly shorter than the relaxation time, and the total time of forward streaming always distinctly longer than the time of reverse flow, it becomes obvious that the populations of collected data cannot be symmetrically distributed in the contraction: streaming contingency tables. It was verified in the four types of control sites examined in present experiments. The results are shown by the diagrams in Fig. 3.

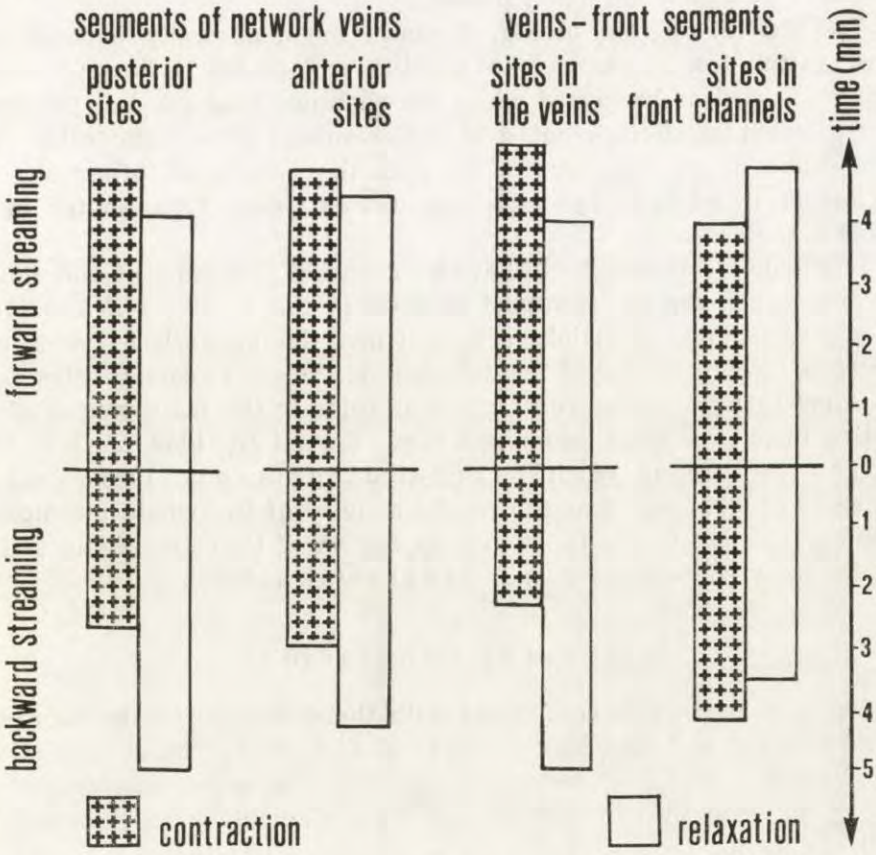

Fig. 3. The total times of forward and backward protoplasm streamings at the four types of investigated control sites, calculated separately for the contraction and relaxation phases

The first three diagrams represent the situation encountered in the veins of the network. The distribution of the contraction and streaming times recorded at all these three sites are very similar one to another. During the relaxation phases the forward and backward streaming times are either equally long or show no more than a few per cent difference in favour of the backward flow. The polarization of the protoplasm transport is almost entirely due to the contraction phases. Roughly speaking, the forward protoplasm flow takes about 2/3 and its backward flow only 1/3 of the total contraction time of the veins. Similar distributions may be demonstrated in the case of data collected earlier in other studies (see Table 5 D–E). It may be considered therefore as well proved that the veins' relaxation is of minor importance in polarizing the streaming direction, whereas the net difference in favour of the forward transport of protoplasm is mainly produced at their contraction phases.

The fourth diagram in Fig. 3 shows the diametrally opposed situation in the frontal channels. Here, the protoplasm is flowing indifferently forward or backward when the channels contract, but the frontally directed streamings get a clear advantage when they relax. This observation is in full agreement with the results of other our experiments (Kołodziejczyk and Grębecki 1983, quoted in the Table 5 D-E).

It should be generalized that the eventual resultant of the shuttle streaming, i.e., the net transport of mass in the direction of locomotion of a plasmodium, is mainly arising when the network of veins contracts and when the small frontal channels relax. It corroborates again the view that the active contraction of veins is the main source of the motive force producing locomotion (e.g., Kamiya 1959, Jahn 1964, Hülsmann and Wohlfarth-Bottermann 1978a and b, Kessler 1982) and that the motile activity of the whole plasmodium is integrated due to the functional opposition of its veins region and its frontal zone (Grębecki and Cieślawska 1978).

General Conclusions

The present results, confronted with the data reported by us earlier (Grębecki and Moczoń 1978, Kołodziejczyk and Grębecki 1982 and 1983) permit to consider now some quantitative relations between the contraction and flow phenomena in plasmodia as well established facts.

The contraction phases, recorded *in situ* in form of the vein's diameter decrease, are always slightly shorter than the relaxation phases

and take in average 48% of the total time. The forward streaming phases are in average 25% longer than the periods of the reverse flow (they take about 55.5% of the total time). Certainly, such a difference is of great importance for the polarization of the net transport of mass in the direction of locomotion. Both these time ratios do not significantly vary depending on the position of the control site or on the external conditions tested up to now.

The contractions and the streamings are both positively correlated in time between two control sites located within the network of veins, but the frontal channels are out of phase. The synchronization of veins is easily deteriorated by external factors (the white light). In general, the synchronism is very imperfect and may be only statistically demonstrated. But the deviations from the synchronous pattern are chaotic and, therefore, they cannot serve as a proof of the existence of any other coordination pattern.

The forward streaming periods in the veins are positively correlated with the local contraction phases. This correlation is also affected by light and changes depending on the position of control sites. It sensibly weakens in the posterio-anterior direction. The forward streaming in any place is also positively correlated with contractions taking place behind it, but not with those occurring further toward the front.

Several facts support our earlier statement (Grębecki and Cieślawska 1978) that the veins region and the frontal zone function in the antagonistic way: (1) The contraction : forward streaming correlation, which is positive in the veins, becomes negative in the frontal channels. (2) The forward streaming in the frontal channels is in the same degree correlated to the contraction of veins lying behind them as to their own relaxation. (3) The net difference in time between the length of forward and backward flow periods, in the veins is established during their contraction, but in the frontal channels during their relaxation.

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ACTA PROTOZOOLOGICA
Vol. 22, No. 1, pp. 19–31 (1983)

Effects of White-red Illumination Changes on the Coordination of Some Motor Functions in Plasmodia of *Physarum polycephalum*

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Received on 10 September 1982

Synopsis. Plasmodia initially kept in uniform white or uniform red light were subsequently exposed to the illumination change, which affected either only their fronts or was limited to the veins' region alone. The illumination changed from white to red or from red to white respectively. The correlation coefficient between the contraction and forward streaming phases was the parameter sensitive in the whole plasmodium, when the stimulus was localized at the front. It became more positive in the veins and more negative in frontal channels after exposing the front to red light, and on the contrary, its anterio-posterior polarization was decreasing when the front was illuminated by the white light. The veins' region seems to be much less sensitive and less selective to the photic stimuli than the frontal zone.

The present state of knowledge allows us to admit that the shuttle streaming in plasmodia of *Physarum* is a hydrodynamic flow which follows the pressure gradients (Kamiya 1959, Jahn 1964), that the pulsation of veins is due to their own local contractile activity (Hülsmann and Wohlfarth-Bottermann 1978a, b) but not a possive response to the protoplasm influx or outflux (Yoshimoto and Kamiya 1978), and that the network of veins and the advancing frontal edge are functionally opposed to one another, because their contraction-expansion phases alternate (Grębecki and Cieślawska 1978). Two postulations may be deduced from these premises: (1) The forward streaming phase should be correlated in time with the contraction phase, positively in the veins and negatively in the frontal channels. (2) The contracting factors should act as negative stimuli and relaxing factors

as positive stimuli when applied to the frontal region, whereas the opposite effects might be expected after their application to the network of veins.

As to the first postulation, a significant but very weak correlation (coefficient $\varphi = 0.16$) between contraction and forward streaming in the middle size veins was found by Grębecki and Moczoń (1978). A second attempt made by us (Kołodziejczyk and Grębecki 1982) demonstrated much higher correlation of both phenomena ($\varphi = 0.35$) in the principal veins of the network kept under more appropriate light conditions. Their correlation in the small channels inside the frontal zone has never been investigated. One of the objectives of the present study was, therefore, to compare the contraction : streaming correlation in the veins and in the front, under different illumination of these regions.

Some data of other authors corroborate the second postulation. The blue light (and white in which the blue is active) may be considered as contracting stimulus, because it increases the force output measured by the double chamber method (Hato et al. 1976) or tensiometrically (Hato et al 1976, Block and Wohlfarth-Bottermann 1981). According to the same authors, the red light decreases the force measured in the double chamber, and has not effect at all in the tensiometric experiments. The negative phototaxis of plasmodia should be therefore expected in response to the blue light and to the white panchromatic illumination due to its blue component. And in fact, such responses were described by Rakoczy (1973), Białczyk and Rakoczy (1974, 1975), Rakoczy (1980) and Block and Wohlfarth-Bottermann (1981). Consequently, the second objective of our present experiments was to test in which way the illumination change from white to red or *vice versa* affects the correlation between the contraction and streaming phenomena, when the change is applied separately either to the frontal zone or to the network of veins. We reported earlier (Kołodziejczyk and Grębecki 1982) that this correlation was improved after reducing the white light intensity over the front.

Material and Methods

Slime molds plasmodia were grown on the wet filter paper and fed on the oatmeals according to Camp (1936). Their fragments were transferred to Petri dishes with 1.5% nonnutritive agar gel and left for 24–36 h in the white or red light, at room temperature. The reorganized, 1–2 days old plasmodia, with well differentiated frontal zones and the network of veins, were used in experiments.

Plasmodia of the first group were placed on the stage of an inverted microscope and illuminated with white light (700–1400 lux) of a photographic enlarger. In the second group of experiments a red filter was put in the optical path of the enlarger. The filter reduced the total light intensity to 50% of its original value. It manifested 50% transmission at 612 nm, practically eliminated the violet, blue, green and yellow components of the white light, but allowed a good transmission in the red and far-red ranges including the wavelengths of 650 nm and 720 nm, which were investigated by previous authors (Hato et al. 1976, Block and Wohlfarth-Bottermann 1981).

The microscope was equipped with the TV camera and monitor. A photoelement connected to the recording system surveyed, through a photographic lens, a field selected on the TV screen. So, the pulsation curves of the examined network veins or frontal channels were plotted automatically on the recorder's chart. A hand-operated device permitted to change the thickness of the plotted curve, and was used to differentiate between the forward and reverse direction of the protoplasmic flow.

Each experiment was run for 3000 s. During the first 1000 s whole plasmodia were evenly illuminated by the white or red light, as described above. Then, by an appropriate movement of the red filter in the enlarger, the illumination was changed from white to red or from red to white respectively, either over the front or over the veins' region, and the recording was continued for another 2000 s.

Observations and records were taken at the middle part of main plasmodial veins or in the small streamlets inside the frontal zone. In total, 160 plasmodia were examined and each type of experiment was repeated 20 times (cf. Table 1). The data for calculations were read out from the records at 1 mm intervals, i.e., at 5 s time lapse, which produced 600 scores for each pair of parameters assessed

Table 1

Distribution and size of the experimental groups depending on the type of initial light conditions character and localization of the illumination change and the site of recording the effects

160 plasmodia investigated	80 initially kept in the uniform white light	40 exposed to the red light at the frontal zone	20	local effects recorded in the frontal channels
			20	distant effects recorded in the network veins
		40 exposed to the red light at the veins' region	20	distant effects recorded in the frontal channels
			20	local effects recorded in the network veins
	80 initially kept in the uniform red light	40 exposed to the white light at the frontal zone	20	local effects recorded in the frontal channels
			20	distant effects recorded in the network veins
		40 exposed to the white light at the veins' region	20	distant effects recorded in the frontal channels
			20	local effects recorded in the network veins

in each experiment. The synchronization rate between the contraction-relaxation cycles of the veins or frontal channels and the shuttle streaming oscillations in them was examined by the χ^2 test. The time relationship between the contraction phase and the forward streaming phase was stated in form of the correlation coefficient $\varphi = \sqrt{\chi^2/N}$, as described in our earlier papers (Grębecki and Moczoń 1978, Kołodziejczyk and Grębecki 1982). The mean values of $\varphi = \pm 0.04$ at the initial light conditions, and $\varphi = \pm 0.03$ after the experimental illumination change, were significant at the level of $p = 0.01$.

Results

The scheme of experimental situations is shown in Fig. 1, the dichotomic subdivisions of the experimental groups and their size in Table 1. In some groups of plasmodia kept under the initial light conditions (Fig. 1, centre of the picture), the illumination of the front was changed from white to red or *vice versa*, without any change affecting the network of veins (left side of the scheme). In other groups the change of illumination type was applied to the veins' region, whereas the frontal zone remained under the initial conditions (Fig. 1, right side). Each type of experiment was repeated in two variants in which the streaming and contraction rhythms were recorded in two different sites: either in the middle segment of one of the principal network veins, or in the small branching channels within the frontal zone. It means that as well the local influence of the illumination change was tested, as its distant effects manifested in the unaffacted parts of plasmodia (Table 1).

Figure 1 indicates that in the second phase of experiments plasmodia were intersected by the white-red illumination borderline in such a way that the posterior part was composed exclusively of the well developed veins and their withdrawing ends. The part called here the front or the frontal zone, was more complex. It comprised the advancing anterior ridge, the continuous protoplasmic layer in which the flow is organized in the small streams (called frontal channels) without differentiation of separate veins, and also the youngest anterior portion of the network of differentiated veins. In the posterior part the records were taken in one of the major veins in the central area of the network, and those concerning the anterior part in the frontal channels, i.e., in the streamlets crossing the continuous protoplasm layer behind the advancing margin of plasmodium.

The produced records of the contractile activity and streaming oscillations allowed us to assess the influence of changing illumination on the mean period of the contraction-relaxation cycles, the time ratios

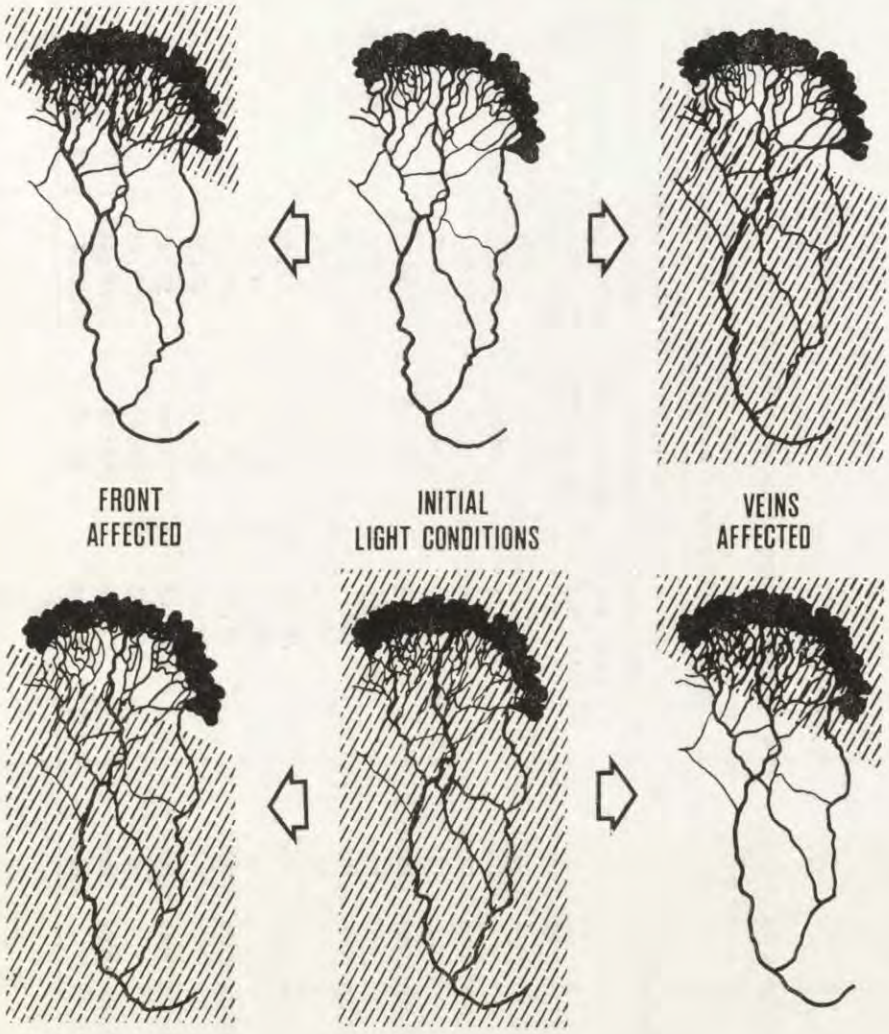

Fig. 1. Scheme of experimental situations. The upper row of drawings shows plasmodia evenly illuminated at the beginning by white light (centre), which were subsequently exposed to the red light either at the front (left) or at the vein's region (right). In the lower row, plasmodia kept initially in uniform red illumination (centre) were exposed to the white light, affecting their fronts (left) or only their networks of veins (right). The dashed areas represent the surface screened by the red filter

of the protoplasmic flow, and the degree of coincidence between the of contraction *vs.* relaxation and of forward *vs.* backward direction contraction and forward streaming. All these data are summarized in Table 2.

The mean periods of the contraction-relaxation cycles oscillated

Table 2

Effects of changing the illumination type upon the contraction and streaming relations

Type of experiment			(A) Front exposed to a change		(B) Veins exposed to a change	
			uniform white illumination turns to red over the front	uniform red illumination turns to white over the front	uniform white illumination turns to red over the veins	uniform red illumination turns to white over the veins
Correlation coefficient φ between the contraction time and the forward streaming phase. It changes:	in the front	from	−0.09	−0.12	−0.02	0
		to	−0.15	−0.09	−0.12	−0.02
	in the veins	from	0.16	0.36	0.21	0.29
		to	0.30	0.22	0.21	0.20
Mean period of the contraction-relaxation cycle. It changes:	in the front	from	85.5 s	88.5 s	90.9 s	89.3 s
		to	78.1 s	81.3 s	79.4 s	83.3 s
	in the veins	from	94.3 s	89.3 s	94.3 s	90.1 s
		to	83.3 s	90.1 s	82.6 s	83.5 s
Contraction : relaxation time ratio. It changes:	in the front	from	48 : 52	48 : 52	50 : 50	47 : 53
		to	50 : 50	47 : 53	49 : 51	48 : 52
	in the veins	from	47 : 53	46 : 54	47 : 53	49 : 51
		to	46 : 54	49 : 51	47 : 53	49 : 51
Forward : backward streaming time ratio. It changes:	in the front	from	57 : 43	56 : 44	55 : 45	56 : 44
		to	55 : 45	53 : 47	57 : 43	55 : 45
	in the veins	from	54 : 46	55 : 45	56 : 44	58 : 42
		to	54 : 46	53 : 47	56 : 44	60 : 40

about 1.5 min approximately, without any significant difference between plasmodia exposed initially either to the white or to the red light. The subsequent experimental illumination changes, which affected either the frontal zone or the veins' region, also failed to produce any appreciable regular effects on the pulsation period. This result may appear strange, because the contraction frequency is increased by positive chemical stimuli (Durham and Ridgway 1976), temperature (Achenbach and Wohlfarth-Bottermann 1980 a, b), and decreased by the blue and white light (Block and Wohlfarth-Bottermann 1981, Wohlfarth-Bottermann and Block 1981). However, the light intensity changes tested in the present study were always below the treshold reported by Block and Wohlfarth-Botterman (1981) at 1500 lux.

The contraction: relaxation time ratio, as well as the forward: backward streaming time ratio, proved to be not sensitive to the differences in the character of initial illumination and to its changes in the course of experiment. Roughly speaking they oscillated closely about 48 : 52 (contraction : relaxation ratio) and 56 : 44 (forward : backward streaming time), which is consistent with the values reported by us elsewhere (Kołodziejczyk and Grębecki 1982, Grębecki and Kołodziejczyk 1983).

On the contrary, the correlation rate between the contraction and the forward streaming phases proved to be a parameter sensitive to the investigated variations of light conditions. The correlation coefficients φ, which are always distinctly positive in the veins' region, were generally higher in plasmodia kept before the experiment in the uniform red light, than in those maintained in the full white illumination. Correlation coefficients in the frontal zone were in both cases slightly negative.

When the frontal zones are now exposed to a change of the illumination type, the φ values change everywhere in the regular manner. They become clearly more positive in the veins' region and more negative in the frontal zone, after changing the illumination of the front to the red one. And *vice versa,* when the illumination of the front alone turns from red to white, the coefficient φ becomes less positive in the veins and less negative in the frontal channels (Table 2, part A) This relationship is demonstrated graphically in Fig. 2. The diagram puts in evidence that the anterio-posterior polarization of φ values becomes better expressed when the front is exposed to the red light, but less pronounced when it is submitted to the white illumination. In the individual curves recorded in the veins (as those shown in Fig. 3 A) it is expressed by more regular contraction and streaming oscil-

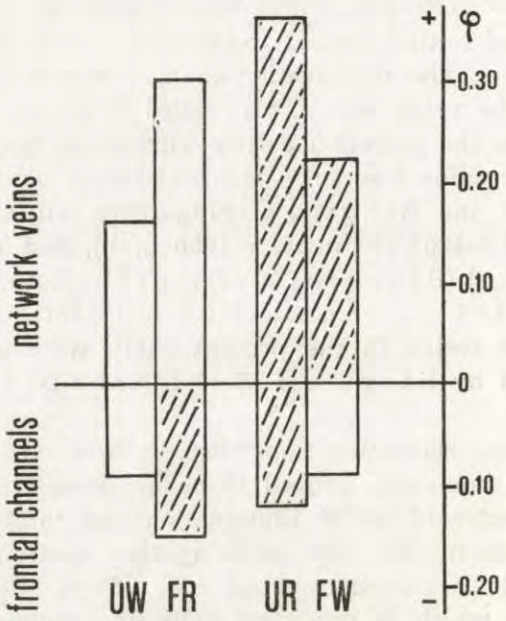

Fig. 2. Polarization of the contraction : streaming correlation coefficients φ which are positive in the veins and negative in the frontal channels. The polarization of φ is less pronounced in the uniform white illumination (UW) than in the uniform red light (UR). It is distinctly increased by exposing the front to red light (FR), but strongly reduced after its exposure to the white illumination (FW)

lations and their better mutual coordination, after the front of plasmodium was exposed to the positive stimulus (red light). Such a favourable effect was found in 60% cases, in 25% it was not very clear, and in 15% rather slightly negative. The deteriorating influence of the negative stimulus (white light) after its application to the frontal zone, leads to more chaotic character of the recorded curves (Fig. 3 B).

The sensitivity of the contraction : streaming correlation coeficient gular effects were manifested only when the frontal zone has been exposed to a change of light conditions. When the same stimuli were applied to the veins' region alone, the produced effects proved to be irregular and difficult to understand. Screening the veins by the red filter had no effect at all, but their exposure to the white light suppressed the contraction: streaming correlation (Table 2, part B).

The sensitivity of the contraction: streaming correlation coefficient to the illumination changing at the front of plasmodium allowed us to deduce that some significant changes in the forward : backward streaming time ratios could also be detected in this experiment, provided that these ratios are calculated separately for the contraction and for

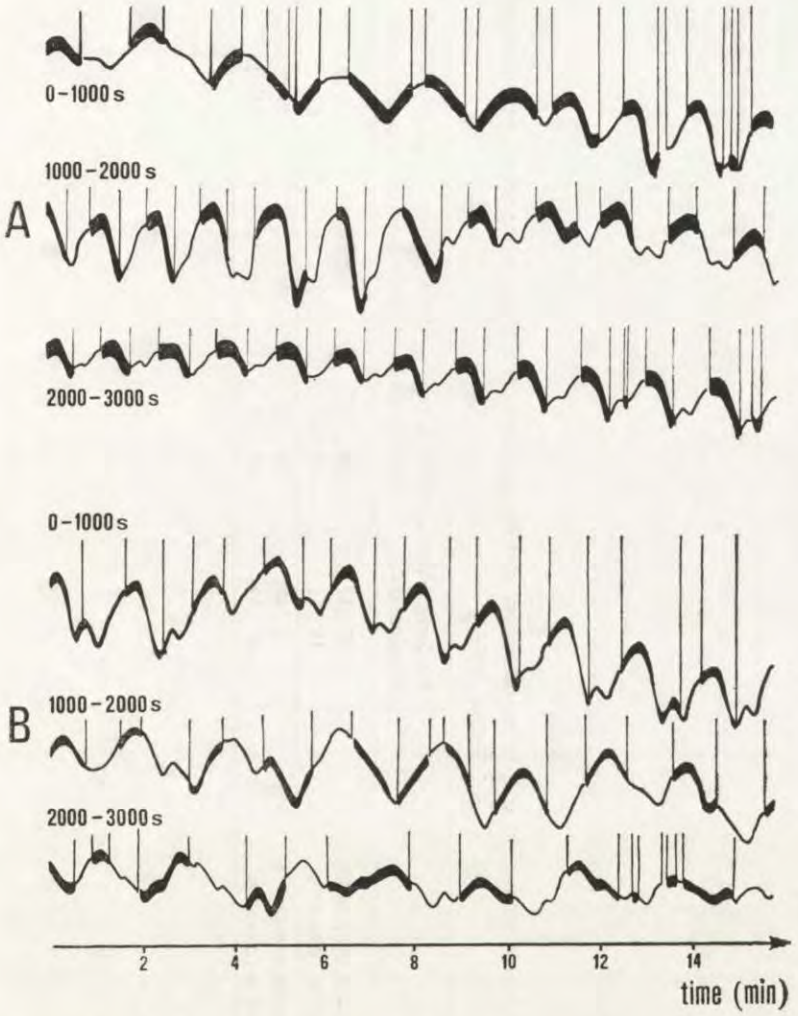

Fig. 3. Examples of the contraction and streaming oscillations recorded in principal network veins, in the plasmodia which were initially kept in the full white light (0–1000 s, in A) or in uniform red illumination (0–1000 s, in B). Note the improved regularity and coordination of both oscillations after exposing the front to the red light (1000–2000 s and 2000–3000 s, in A), and their deterioration after illuminating it with the white light (1000–2000 s and 2000–30000 s, in B). The declining arms of the oscillation curves correspond to the contraction phases, and the heavy line segments to the forward streaming periods

the relaxation periods (instead of producing total figures as in the Table 2). The results of such analysis are shown in the Table 3. They clearly demonstrate that in the network of veins the streaming is strongly polarized in favour of the forward direction when the veins contract, but its inverse polarization during the relaxation is distinctly less pro-

Table 3

Forward : backward streaming time ratios during contraction and during relaxation, at different illumination types, in the veins' region and in the frontal zone

Illumination type		Uniform white illumination...	... turns to red over the front	Uniform red illumination...	... turns to white over the front
Forward : backward streaming time ratio recorded in the veins	during contraction	63 : 37	70 : 30	74 : 26	65 : 35
	during relaxation	45 : 55	40 : 60	39 : 61	43 : 57
Forward : backward streaming time ratio recorded in the front	during contraction	52 : 48	48 : 52	49 : 51	49 : 51
	during relaxation	61 : 39	62 : 38	62 : 38	57 : 43

nounced. In the frontal zone channels, on the contrary, the difference between the forward and backward streaming time is almost insignificant during contraction, but the forward flow gets the advantage during the relaxation. These relationships seem to be reinforced when the front is screened by the red filter, and get weaker after its exposure to the white illumination. It is also worthy to note that the illumination changes which are applied to the frontal zone, exert much more pronounced distant effects in the veins, than directly in the frontal channels.

Discussion

It may be concluded in general that the red illumination employed in this study distinctly improves the coordination of the motile phenomena in the whole plasmodium, when it is applied to the frontal region alone (whereas the network of veins remains in the white light). This improvement is manifested by a much better correlation in time of the forward protoplasm streaming with the contraction phase in the large network veins and with the relaxation (expansion) phase in the small frontal channels. It means that the motor polarization of the whole plasmodium, expressed by the functional opposition between the veins and the front (Grębecki and Cieślawska 1978), is enhanced. It should be expected therefore, that under such conditions the net transport of mass in the frontal direction becomes probably more regular and more efficient. And on the contrary, when the panchromatic white light with its active blue component is applied to the frontal region alone (whereas the veins remain in the red illumination), the motor coordination of the whole plasmodium is deteriorating. The functional opposition of the network veins and the frontal channels decreases, which should certainly disfavour the locomotion in the former direction. Therefore, the coordination and polarity changes described here, fit in very well with the findings of other authors who demonstrated that freely moving vegetative plasmodia are photonegative to the white light (Rakoczy 1973, Białczyk and Rakoczy 1974, 1975) and plasmodia examined in the double chamber are photonegative to the blue and photopositive to the red wavelengths (Hato et al. 1976).

We expected that the illumination changing from white to red, or *vice versa,* over the network of veins alone will produce the effects exactly opposed to its application to the frontal region. But the results proved to be insignificant with the red and contrary to our expectations with the white light. Perhaps, the red is less effective in the veins than in the front, and the white (or blue) disturbs the contraction:

streaming correlations independently of its application site. In the experiments of Block and Wohlfarth-Bottermann (1981) the blue and white light increased the force output of protoplasmic strands, but the red of the same intensity did not affect the tensiometric response.

On the other hand, the fact that the positive or negative stimuli applied to the front alone exert the unequivocal and regular influence on the motor coordination of the whole plasmodium, whereas their application to the veins' region brings rather uncertain and confused effects, may be nevertheless significant and important from the behavioural point of view. It is obvious that during the locomotion the front first meets new environmental situations which act as tactic stimuli, and it must produce an appropriate response. Therefore, the front may be in fact more sensitive and more selective to stimuli, and the direct effects of their action on the front may influence the behaviour of the more distant posterior parts of plasmodium.

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ACTA PROTOZOOLOGICA
Vol. 22, No. 1, pp. 33–42 (1983)

Regeneration of *Tetrahymena* Cilia Under the Influence of a DNA-ligand: 4,6-diamidine-2-phenylindole (DAPI)

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Received on 27 July 1982

Synopsis. Starved, deciliated cells of *Tetrahymena pyriformis* recover their mobility after 40 min after trauma. The cells incubated with DAPI during whole starvation period (20 h) restore their swimming ability after 70 min. On the other hand, cells incubated with the drug for only 40 min before deciliation, regain their mobility after 40 min, similarly to the controls, though their macronuclei display the presence of the ligand within them, manifested by fluorescent complexes of DAPI-DNA. However, if the latter cells were regenerated their cilia in DAPI-supplemented medium for 120 min, and once more deciliated, mobile cells were noted after 70 min. Incorporation of ^{3}H-uridine during cilia regeneration into 20 h DAPI-treated cells reveals that DAPI inhibits RNA synthesis induced by deciliation. The possible mechanism of the action of DAPI delaying recovery of cell mobility is discussed.

Deciliated *Tetrahymena* cells recover their mobility in about 40–60 min, though at this moment the length of the cilia is only partially restored (Rosenbaum and Carlson 1969, Guttman and Gorovsky 1979, Bird and Zimmerman 1980). According to Williams (1975) and Bird and Zimmerman (1980) full restoration of the length of cilia takes about 120 min. Very little, if any, amino acid incorporation into regenerating cilia was observed (Nelsen 1975), and thus, it was suggested that mainly the intracellular pool of proteins, especially tubulin, is utilized for reconstruction of cilia (Rosenbaum and Carlson 1969, Rannestad 1974, Guttman and Gorovsky 1979). However, in deciliated cells a steady increase of the amount of polysomes was observed very early after amputation (Guttman and Gorovsky 1979, Bird and Zimmerman 1980), owing to which, the synthesis of proteins, particularly those of high

molecular weight was detected (Guttman and Gorovsky 1979, Bird and Zimmerman 1980, Skriver and Williams 1980). Therefore, it was concluded that the newly synthesized proteins (tubulin) are mainly utilized for supplementation of the cellular pool, but not for current reconstitution of cilia. Only when this pool is insufficient — for example, after long lasting cell starvation — the newly synthesized proteins are also used in reconstitution of cilia (Guttman and Gorovsky 1979).

Contrary to the observed early synthesis of proteins, the synthesis of total RNA is not significantly augmented at least during the first hour of cilia regeneration as compared with nondeciliated cells. The only difference noted as soon as 5 min after deciliation seems to concern the steady increase in poly(A)-containing RNA, particularly messenger RNAs for α- and β-tubulin (Marcaud and Hayes 1979). A significant rise in the activity of RNA synthesis is noted as late as the second hour of cilia regeneration, that is, when the cells regain their mobility (Guttman and Gorovsky 1979, Marcaud and Hayes 1979).

In the present study we have used a ligand of DNA—4,6-diamidine-2-phenylindole (DAPI). Since it was reported that DAPI is responsible for inhibition of RNA synthesis, *in vitro*, (Skoczylas 1980 b), it seemed interesting to study whether the process of cilia regeneration, which is known to induce RNA synthesis would be in any way impaired by the presence of this drug. Fluorescent DAPI complexes with DNA (Williamson and Fennell 1975, Kapuściński and Skoczylas 1978) can be used for staining *in vivo*, the cellular structures containing DNA (Williamson and Fennell 1975, Skoczylas 1980 a, Prantera et al. 1981). Therefore, the appearance of the ligand in the macronucleus of *Tetrahymena pyriformis* can be easily observed under the fluorescent microscope.

Material and Methods

Cells of the amicronucleate strain of *Tetrahymena pyriformis* GL grown at 27°C on 1% proteose peptone and 0.1% yeast extract were used.

On the 4th day after inoculation into growth medium, the cells were harvested by gentle centrifugation (500 g) and resuspended in 50 mM Tris-HCl buffer, pH 7.2, in concentration of about 4×10^5 cells per mililiter. After 20 h of starvation in this buffer, the animals were deciliated by calcium shock according to Guttman and Gorovsky (1979). Deciliated cells were immobile, as checked under the microscope. Recovery of mobility of the cells transferred into fresh starvation buffer indicated an advanced process of cilia regeneration.

The dose of a DNA ligand — 4′,6-diamidine-2-phenylindole · 2HCl (DAPI)[1] was chosen experimentally by comparing the influence of various drug concentrations on population development and the time necessary to visualize the macronucleus under the fluorescent microscope (ERGAVAL, Zeiss). As seen in Fig. 1, DAPI in

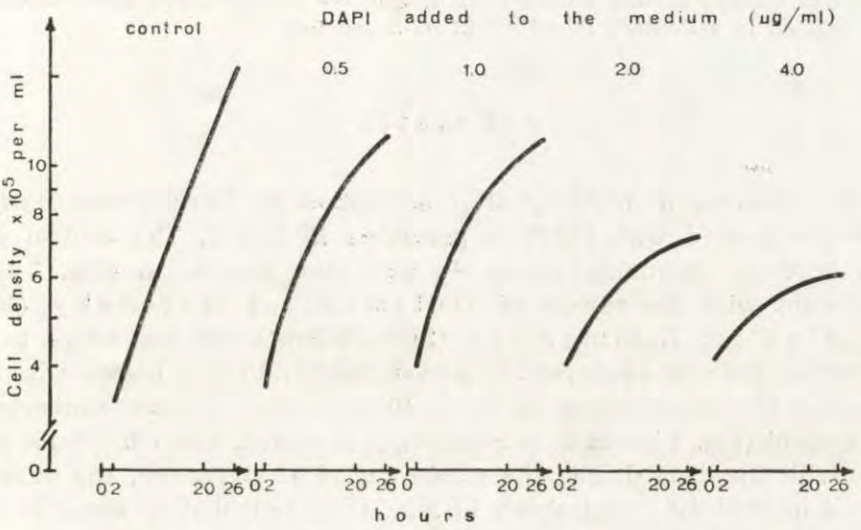

Fig. 1. Growth of *Tetrahymena pyriformis* GL population in 0.5, 1.0, 2.0 and 4.0 μg of DAPI per ml on proteose pepton and yeast extract medium. The cells were counted at 0, 2, 20 and 26 h after drug addition. From parallel samples, every 10 min the cells were observed in a fluorescent microscope. Fluorizing macronuclei were found in cells incubated with 4 μg/ml of DAPI after 20 min, 2 μg/ml after 30 min, 1.0 μg/ml after 40 min and 0.5 μg/ml after 60 min

concentrations of 0.5 and 1.0 μg/ml only slightly impaired population development. However, DAPI in solutions of lower concentration needs more time to be visible in the macronucleus. Thus, in experiments on cilia regeneration we used DAPI in concentration of 1.0 μg/ml as having a very little inhibitory effect on population development and penetrating into the macronucleus quicker than DAPI of 0.5 μg/ml concentration — 40 and 60 min, respectively.

In experiments on cilia regeneration DAPI was added to the culture for the whole period of starvation or only for the last 40 min before deciliation. As noted above, 40 min incubation with the drug was sufficient to find the ligand in the macronuclei by means of fluorescence technique. Cilia regeneration was carried out in DAPI-free Tris buffer. When two consecutive deciliations were performed, DAPI was present in the culture medium during 160 min: it was added for 40 min before first amputation and remained for 120 min of reciliation period until the second trauma. As reported by Williams (1975) and Bird and Zimmerman (1980), a 120 min period is necessary for cilia to attain full length.

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[1] The author wishes to express sincere thanks to Dr. B. Skoczylas for supplying DAPI.

RNA synthesis was measured by the incorporation of ^{3}H-uridine (specific activity 44 Ci/mmole, Ammersham) into acid-insoluble material. The cell samples, taken consecutively at 20 min intervals in the course of cilia regeneration, were incubated with the isotope in concentration of 2 μCi/ml during 20 min. Incorporation was stopped by addition of an equal volume of ice-cold 10% TCA. The precipitated material was collected on Synpor No. 6 (Chemapol, Czechoslovakia), and counted in a Packard liquid scintillation counter.

Results

The recovery of mobility after deciliation of *Tetrahymena pyriformis* cells treated with DAPI is presented in Fig. 2. The control cells recover their swimming ability 40 min after deciliation (Fig. 2 a) in agreement with the results of Guttman and Gorovsky (1979) and Bird and Zimmerman (1980). When DAPI was added to the culture at the end of starvation period, that is 40 min before cilia amputation, the cells started to move 40 min after trauma, similarly to the control (Fig. 2 b and a, respectively). However, when the cells were kept with the drug during the whole period of starvation, the duration of cell immobility lasted about 70 min. (Fig. 2 c), that is about 30 min longer than in the two above described experiments a and b.

The recovery of cell mobility studied in two consecutive deciliations was not disturbed by the presence of DAPI in the regenerating medium after the first amputation of cilia (Fig. 3b). This resembles the restoration of the swimming ability of cells not incubated with the drug (Fig. 2 a) and those incubated with DAPI for the last 40 min before deciliation (Fig. 2 b). When the regenerating cells were kept in DAPI-enriched medium until the second amputation — 120 min after the first one — the second recovery of cell mobility was delayed ca. 30 min as compared with the control (Fig. 3 b and a, respectively), resembling the appereance of swimming cells in sample treated with DAPI for 20 h (Fig. 2 c). However, if the cells were treated with DAPI just before cilia amputation, as presented in Fig. 2b, but for the same period of time as in the case of two consecutive deciliations — that is 160 min — there was no delay in the restoration of cell mobility (not shown).

Incorporation of ^{3}H-uridine measured in successive 20 min intervals in the course of cilia regeneration into treated with DAPI cells for long period and those never incubated with the drug (variants c and a in Fig. 2, respectively) is presented in Fig. 4. It is seen that RNA synthesis remains nearly at the same level in all DAPI-treated cells. The incorporation of ^{3}H-uridine into the control cells is more variable among all studied samples and in samples 1–3 is similar to that of DAPI

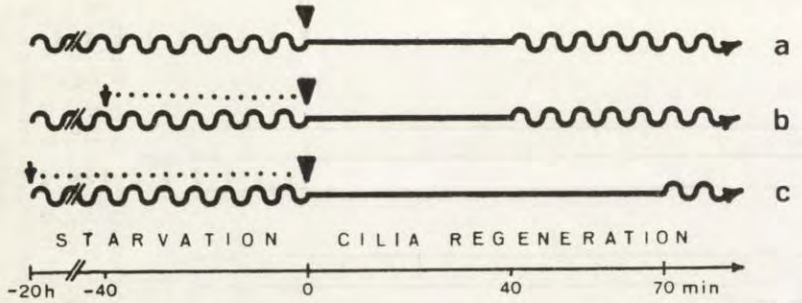

Fig. 2. Recovery of mobility in *Tetrahymena pyriformis* GL treated with DAPI (1.0 μg/ml) before deciliation. *Abreviations*: straight line — immobile cells, wavy line — mobile cells, small arrow — addition of DAPI to the culture, dotted line — DAPI presence in medium, triangle — moment of cilia amputation

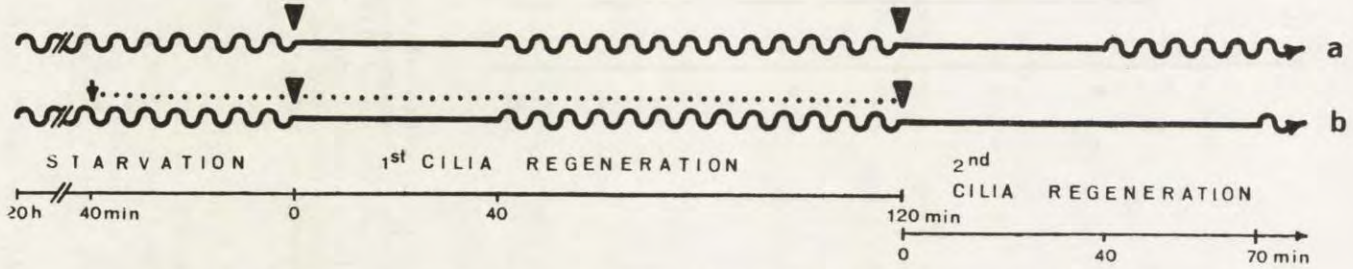

Fig. 3. Recovery of *Tetrahymena* cell mobility in two consecutive deciliations in the presence of DAPI (1.0 μg/ml). Abreviations as in Fig. 2

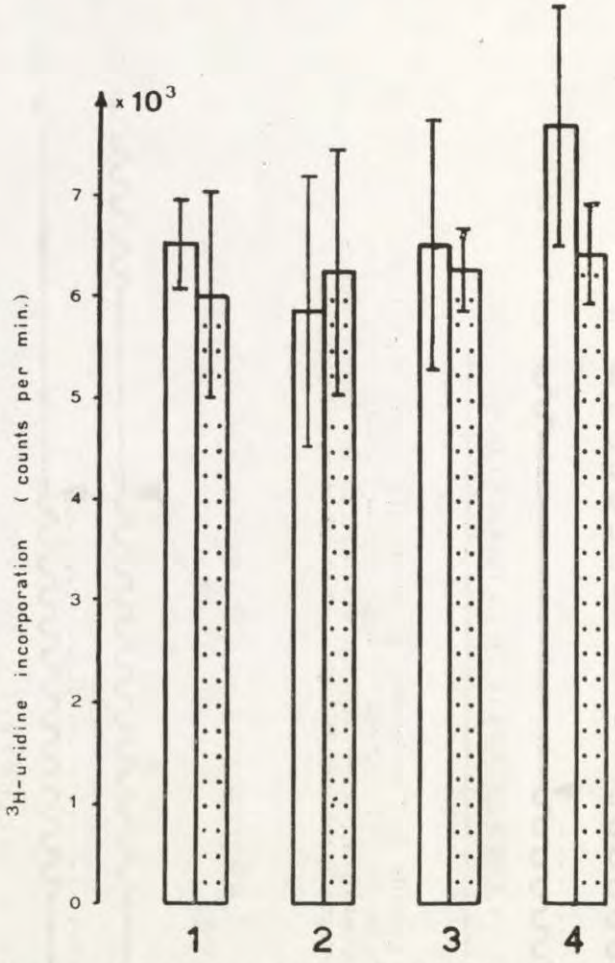

Fig. 4. Incorporation of ^{3}H-uridine into 20 h DAPI-treated cells during cilia regeneration. *Abreviations*: empty bar — incorporation of control cells, dotted bar — incorporation of DAPI-treated cells, calculated per 10^5 cells. Cell samples were taken every 20 min in the course of cilia regeneration for 20 min incubation with isotope (2 μCi/ml). Bar 1 — sample incubated with isotope between 0–20 min of regeneration, bar 2 — between 20–40 min, bar 3 — between 40–60 min, and bar 4 — between 60–80 min

treated ones. The highest activity of RNA synthesis of controls is noted between 60–80 min of reciliation. This is consistent with the data reported by Guttman and Gorovsky (1979) and Marcaud and Hayes (1979) on increase of RNA synthesis at the beginning of the second hour of cilia regeneration. Statistical analysis by means of t-student test showed that observed in sample 4 inhibition of RNA synthesis of DAPI-treated cells *vs.* nontreated ones is significant with the probability p_ε (0.9–0.95).

Discussion

As shown in this paper, short incubation with DAPI before deciliation, as well as the presence of the drug during cilia regeneration after such short treatment, has no influence on recovery of cell mobility (Fig. 2 a, b and 3 a, respectively). The cells begin to swim 40 min after cilia amputation, similarly to the control cells, resembling the restoration of the swimming ability reported by other workers (Rosenbaum and Carlson 1969, Rannestad 1974, Williams 1975, Guttman and Gorovsky 1979, Bird and Zimmerman 1980). A prolongation of the period of cell immobility was noted only when DAPI was present in the medium either for the whole 20 h of starvation or for 160 min, when two consecutive deciliations were performed (Fig. 2 c and 3 b, respectively).

It is known from the earlier studies that regeneration of cilia in *Tetrahymena* proceeds on an account of the intracellular protein pool (Rosenbaum and Carlson 1969, Rannestad, 1974, Nelsen 1975, Williams 1975, Guttman and Gorovsky 1979). Therefore, prolongation of the cilia regeneration is presumably the result of insufficiency of the pool.

The depletion of the intracellular pool during starvation is well documented by the decreased ability of cell division. Guttman and Gorovsky (1979) reported that during 20 h of starvation the cells are able to divide only once. Our observation reveal that only 50% of cells divided, whereas in the presence of DAPI less than 20% of cells manage to complete one division (Krawczyńska, unpublished), what is presumably connected with the inhibition of the template by the drug. It was observed *in vitro*, that replication is blocked by the presence of DAPI (Mildner and Chandra 1979). On the other hand, the inhibition of the DNA-template seems to be confirmed by a slightly lower incorporation of ^{3}H-uridine into long-time DAPI-exposed cells (Fig. 4). This inhibition persists even at the begining of the second hour (sample 4 in Fig. 4), while RNA synthesis of the control is augmented similarly to the observation of Marcaud and Hayes (1979) and Guttman and Gorovsky (1979).

It was reported that DAPI forms two kinds of bonds with DNA. In a low molar ratio of DAPI to DNA — for calf thymus not exceeding the value of 0.025 — a very strong fluorescent complexes are formed, specific for AT base pairs. At higher DAPI concentrations, besides fluorescent, much weaker and probably ionic in their nature nonfluorescent complexes have been observed (Kapuściński and Skoczylas 1978, Kapuściński and Szer 1979). It was found that calf

thymus DNA accessibility to *E. coli* RNA polymerase was inhibited by DAPI used in such high concentrations only (Skoczylas 1980 b).

In *Tetrahymena pyriformis* treated with DAPI for a long and short time, the macronuclei shine clearly under the fluorescent microscope, revealing the presence of DAPI fluorescently complexed with DNA. The prolongation of cilia regeneration, as presented in this paper, was found in cells treated for a long time with DAPI.

Taking both these effects into account we can suggest that after long time exposure more drug penetrates into the macronucleus forming not only fluorescent complexes with DNA, but also nonfluorescent ones. It should be emphasized that as well during regeneration of cilia as even several hours after completion the process of reciliation, the macronuclei of *Tetrahymena* are still fluorescent in DAPI-free medium, revealing the persistence of fluorescent complexes of DAPI with DNA. Thus, presumably not the fluorescent but rather nonfluorescent complexes DAPI—DNA influence the process of reciliation.

It should be mentioned that such time dependence of the action of DAPI was reported by Prantera et al. (1981) who found that after longer exposure (5 or 16 h *vs.* 1 or 3 h) of human leucocytes to DAPI, more drug penetrates into the cells.

However, the simple time relation of the DAPI-template inhibition is rather insufficient to explain the delay of cilia regeneration since the presence of the drug for 160 min before deciliation does not disturb *Tetrahymena* reciliation. As known, at this time the transcriptional activity of 20 h starved cells remains at a very low level (Guttman and Gorovsky 1979). On the other hand, in experiments with two consecutive deciliations, a 160 min of DAPI presence was effective in the delay of cilia regeneration. It was reported that deciliation induces RNA synthesis (Guttman and Gorovsky 1979 and Marcaud and Hayes 1979). Therefore, we suggest that just after the first deciliation RNA synthesis was inhibited by the presence of DAPI. In the case of cells starved in the presence of DAPI for 20 h, such inhibition could take place during the last cell division.

It is well documented now, that for initiation of m-RNA synthesis on the ovalbumin and histone H2A genes, specific AT-rich sequences are required (Tsai et al. 1981, Grosschedl and Birnstel 1981). If such sequences are also important for initiation of transcription of tubulin m-RNA in *Tetrahymena*, it may be supposed that these sites could be easily blocked by the presence of DAPI — AT-specific ligand of DNA.

On the other hand, DAPI can act at an other than DNA-template level. The ionic bonds of the drug to RNA were also postulated (Ka-

puściński and Skoczylas 1978, and Kapuściński and Szer 1979). Therefore, it is not excluded that the binding of DAPI to messenger RNA during its processing and/or transport into the cytoplasm may disturb the completion of the protein pool and in turn disturb the restoration of cilia.

A nonspecific effect of the action of DAPI with other than nucleic and molecules cannot, however, be excluded.

ACKNOWLEDGEMENTS

The author is greatly indebted to prof. A. Przełęcka for her advices in preparing the manuscript and to Mrs. D. Kucharczyk for her excellent technical assistance, and to mgr ing. D. Auriga for help in statistical analysis.

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ACTA PROTOZOOLOGICA
Vol. 22, No. 1, pp. 43–53 (1983)

Life Cycle of *Trypanosoma avium bakeri* and Its Host-specificity

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Received on 28 July 1981

Synopsis. The present paper deals with an outline description of the life cycle of *Trypanosoma avium bakeri* in vertebrate and in vertebrate hosts and the results of some cross transmission experiments of the parasite. An attempt will be made here to clarify the taxonomic problems of avian trypanosomes in the light of the above observation.

The taxonomic situation of the avian trypanosome is confusing. No detailed work has been reported on the cross transmission of an identified avian trypanosome, although such experiments bear a good deal of importance towards the understanding of the taxonomy of the group, Baker (1956 b), Levine (1961). Some of the earlier workers have reported that a trypanosome of a house sparrow can infect different birds and mammals, David and Nair (1955), and Grewal et al. (1957). But they have neither identified the species of trypanosome nor have they given any morphological details. Baker (1956 a) and Singh et al. (1956), however, have registered some observations on the morphological variations of the avian trypanosomes in a few experimentally infected birds. Bennett (1970) isolated some morphologically similar *T. avium* from different hosts. According to him, the flagellates have more specificity for the invertebrate than the vertebrate host. Molyneux and Gordon (1975) reported that no cross immunity exists between the three species of avian trypanosomes viz. *T. corvi*, *T. bouffardi* and *T. everetti*. Baker (1975) reviewed the situation and he suggested that *T. avium* should be restricted to parasites of *Strigiformes* (Owls) in the old world.

Paper presented on July 9 at the Symposium A and CPS "Variation, Life Cycles, Systematics and Phylogeny of *Protozoa*" of VI International Congress of Protozoology, Warszawa, Poland, 5—11 July 1981.

The above background clearly indicates that the problem of taxonomy of avium trypanosomes is still confusing. In the present work an attempt has been made to put forward some more facts on cross transmission of the avian trypanosome with a view to understand their taxonomy. Here the behaviour of the trypanosome in experimentally infected birds belonging to 10 different orders has been presented. Variation in size, morphology and other biological features have been studied. Such variations may occur in natural infection as well.

Although several different species of trypanosomes have been recorded from various birds on the basis of morphological and mensural features, there may not be so many distinctly different species. On the other hand, it is likely that fewer species exist in different birds and the rest are synnonymous as suggested by Levine (1961).

Materials and Methods

Avian trypanosomes constituted the material for the present study. During the month of October and November, blood from twenty adult red whiskered bulbuls (*Pycnonotus jocosus*) was examined on three different occasions, for naturally occurring trypanosomes. After the examination, 15% of the birds were found to be naturally infected with trypanosomes. Living trypanosomes occurring in the peripheral blood were studied in cover glass preparations. For detailed observations, smears of blood, lung, heart, spleen, liver kidney, brain and bone-marrow were used. The smears were air-dried, fixed in undiluted Leishman's stain for one minute and then Giemsa's stain (pH 7.2) was gently added.

Cross transmission experiments were carried out with 15 species of birds belonging to 10 different orders and one species of albino rat. The names of the birds with their respective orders are listed below:

Order — *Galliformes*

Newly hatched chick of white leghorn — *Gallus* sp.
Black breasted quail — *Coturnix coromandelicus* (Gmelin)

Order — *Columbiformes*

Domestic pigeon — *Columba livia intermedia*

Order — *Passeriformes*

Red vented bulbul — *Molpestes carfer* (Linn)
Common mynah — *Acridotheres tristis* (Linn)
Pied mynah — *Sturnopastor contra* (Linn)
Grey headed mynah — *Sturnia malabarica* (Gmelin)
Spotted munia — *Uroloncha punctulala* (Linn)

Order — *Psittaciformes*

Blossom headed parakeet — *Psittacula cyanocephala* (Linn)

Order — *Cuculiformes*

Koel — *Eudynamis scolopaceus* (Linn)

Order — *Strigiformes*

Spotted owllet — *Athene brama* (Temminck)

Order — *Anseriformes*

Duckling — *Anas boschas*

Order — *Ciconiiformes*

Little Egret — *Egretta gazzetta* (Linn)

Order — *Piciformes*

Crimson breasted barbet — *Megalaima haemacephala*

Order — *Coraciiformes*

Blue jay — *Coracias bengalensis* (Linn)

Usually six birds of each species were subjected to infective inoculation during the cross transmission experiments.

Before the inoculation of the trypanosome, the birds and rats were tested for naturally occurring trypanosomes by culture of their blood in N.N.N. medium.

Citrated blood of red whiskered bulbul, containing a large number of trypanosomes, was primarily inoculated in young pigeons, day old chicks and clean red-whiskered bulbuls.

The strain of trypanosome was subsequently maintained in pigeons in the laboratory through needle passage for convenience. All other species of birds used in this experiment were inoculated with trypanosomes from the experimentally infected pigeons. The volume of citrated blood varied according to the size of the birds from 0.5 ml to 2 ml.

Host Specificity and Cross Transmission Experiments

The infection was transferred by blood inoculation to fifteen chicks, twenty five pigeons, nine black breasted quails, six red whiskered bulbuls, six red vented bulbuls, six common mynahs, six pied mynahs, six spotted munias, six grey headed mynahs, four cattle egrets, four koels, four spotted owllets, six ducklings, six blossom headed parakeets, six crimson breasted barbets and three roller birds.

Results

Morphology of the Parasite

Trypanosoma avium bakeri was described from naturally infected red-whiskered bulbul (*Pycnonotus jocosus*) Chatterjee and Ray (1971). This trypanosome resembled *Trypanosoma avium* Danilewsky, 1885 in general morphological features but it was larger than the latter in all mensural features (Table 1).

Table 1

Average measurements (in μm) of *Trypanosoma avium* described from different birds as compared to that of *T. avium bakeri*

	T. avium described by Baker (1956) from naturally infected black bird, *Tardus merula merula*	*T. avium* described by Baker (1966) from experimentally infected canary, *Serinus canarius*	*T. avium* described by Grewal (1963) from naturally infected white throated munia, *Uroloncha malabarica*	*T. avium bakeri* described in the present paper from naturally infected red-whiskered bulbul *Pycnonotus jocosus*
Length of the body (excluding free flagellum)	52.1	47.7	40.6	61.5
Length of free flagellum	6.2	7.2	7.0	16.5
Total length of flagellum	56.0	52.9	43.5	80.5
Posterior end to centre of kinetoplast	14.7	14.6	10.7	10.5
Posterior end to centre of nucleus	24.9	24.6	19.5	25.0
Breadth at level of nucleus	5.5	5.7	6.2	7.0
Nucleus (opposite diameters)	4.9×3.0	4.2×2.4	4.7×3.1	8.0×6.0
Kinetoplast (opposite diameters)	1.0×0.8	1.1×0.7	0.6×0.5	1.5×2.2

Division Stages in the Vertebrate Hosts

Division stages of the avian trypanosome were studied in the peripheral circulation and bone marrow of the naturally infected red-whiskered bulbul and experimentally infected pigeon. The parasite multiplied by longitudinal binary fission and two types of plasmotomy. In the first type of plasmotomy, elongated bi or trinucleated trypo-

mastigote forms gave rise to epimastigote (crithidial) forms. The kinetoplasts of such forms did not divide synchronously with the multiplication of the nucleus (Fig. 1).

Fig. 1. Schematic drawings of the division stages of *T. avium bakeri* ssp. nov, in the vertebrate host. A — trypomastigote form, a — trypomastigote form undergoing binary fission, b — two young trypomastigotes after the division, a′-c′ — irregular outlined trypomastigotes undergoing nuclear and kinetoplast division, d′ — amastigote forms, e′ — proepimastigote forms, f′ — groups of epimastigote forms, g′ — young trypomastigote form, a″ — binucleate trypomastigote, b″ — trinucleate trypomastigote (each is accompanying a kinetoplast), c″ — trypomastigote with three nuclei and one kinetoplast, d″ — epimastigote forms, e″ — young trypomastigote form

In the second type of plasmotomy, a trypanosome assumed a boat-shaped appearance or became irregular in outline (Fig. 2) and showed two, three, four or sometimes eight nuclei lying close to one another. Such forms were found in the bone-marrow which usually possessed a single kinetoplast; although forms devoid of kinetoplasts or with more than one kinetoplast were also encountered. The cytoplasm of these trypanosomes gave an impression of undergoing cytotomy. As

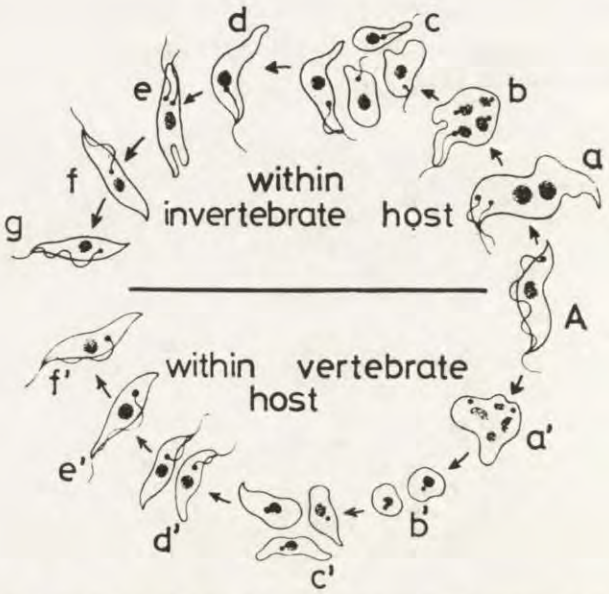

Fig. 2. Schematic drawings of the different stages of division of *T. avium bakeri* within the invertebrate and vertebrate hosts. a–g — different stages within the mosquito, *Aedes albopictus*, A — Trypomastigote form, a — Trypomastigote seen in the fore-gut of mosquito in the early hours. Division of nucleus and kinetoplast has been started, b — trypomastigote with four nuclei and four kinetoplasts, c — large epimastigote forms in groups, d — epimastigote form, e — epimastigote form undergoing binary fission, f — epimastigote form after the binary fission, g — metacyclic form seen in the hind gut of the mosquito, a'-f' — different stages within the avian hosts, a' — irregular outlined trypomastigote found in the bone marrow, with multiple nucleus and kinetoplast, b' — amastigote forms found in the bone marrow of avian host, c' — proepimastigote forms, d' — epimastigote forms, e' and f' — young trypomastigote forms

a result, two or three round or elliptical amastigote forms (leishmanial bodies) were often observed in the bone-marrow smears. These amastogotes changed into proepimastigotes before becoming epimastigotes. The epimastigotes subsequently developed into young trypomastigotes.

Division Stages in the Invertebrate Host and Subsequent Transmission to Birds

T. avium bakeri is known to undergo morphogenetic changes in gut of *Aedes albopictus*. Epimastigotes are produced by multiple fission within 24 h and then the division takes place by binary fission. Conversion of epimastigotes to metacyclic trypomastigotes occurs 48 h after the infective feed. Metacyclic forms are seen only in the mid and hind guts of mosquitoes.

Inoculation of the developing forms, collected from the hind guts of the infected mosquitoes produced infection in clean birds. A few drops of suspension of flagellates, collected in citrated saline from the mid and hind guts of 24 mosquitoes, 60 h after feed, were placed on the sacrified skin of pigeons and produced infection in them. Flagellates collected from faecal samples of mosquitoes, after 60 h of feed, were injected intraperitoneally into pigeons. Trypanosomes appeared within 5 days in the peripheral blood. Flagellates inoculated intramuscularly into pigeons also resulted in patent parasitaemia about 6 days later.

Cross Transmission Experiments

Cross transmission experiments showed that the trypanosome had little host restriction so far as the avian hosts were concerned. They were able to infect fifteen species of birds belonging to ten different orders, viz. *Galliformes*, *Columbiformes*, *Passeriformes*, *Psittaciformes*, *Cuculiformes*, *Strigiformes*, *Anseriformes*, *Ciconiformes*, *Piciformes* and *Coraciformes*. But the trypanosome failed to infect albino rats when they were inoculated along with the birds.

The behaviour of the trypanosome in the different experimentally infected birds indicated that the susceptibility of the trypanosome varied from bird to bird. From the histograms of the mensural data (Fig. 3) and from records of biological features of the trypanosome in

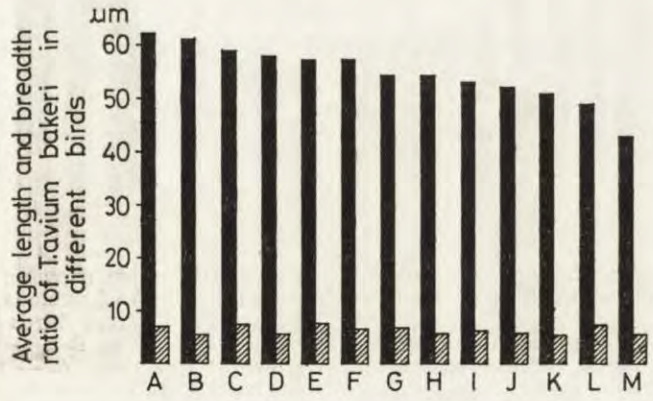

Fig. 3. Histogram showing average length and breadth ratio of *Trypanosoma avium bakeri* in different birds. (A — from natural infection, B–M — from experimental infection). A — red whiskered bulbul, B — spotted owllet, C — blossom headed parakeet, D — crimson breasted barbet, E — pigeon, F — blue jay, G — koel, H — spotted munia, I — little egret, J — chick, pied mynah and domestic duck, K— black breasted quail, L — common mynah, M — red vented bulbul

various birds considerable variations were recorded. The maximum length of the trypanosome noted in naturally infected red-whiskered bulbul was 61.5 μm and experimentally infected spotted owllet was 60.5 μm. The minimum average length of the body recorded in trypanosomes from red vented bulbul was 43.5 μm. The breadth of the body of the trypanosome in different hosts also showed certain variation. The maximum breadth of the body recorded in the trypanosomes from pigeon, common mynah and parakeet was 7.5 μm. The minimum breadth noted in the trypanosomes from quail, red vented bulbul, spotted owllet and barbet was 5.5 μm. The area of the nucleus and kinetoplast (Fig. 4) was also found to vary among the trypanosomes collected from different experimentally infected birds. The area of the nucleus was found to be maximum (about 41.5 sq. μm) among the trypanosomes in common mynah and parakeet and the minimum among the trypanosomes of spotted owllet (17.66 sq. μm) and crimson breasted barbet (about 18.84 sq. μm). Among the trypanosomes, the area of kinetoplast (Fig. 5) was maximum (2.35 sq. μm) in pigeon and common mynah and minimum in the trypanosomes of grey headed mynah (about 0.35 sq. μm).

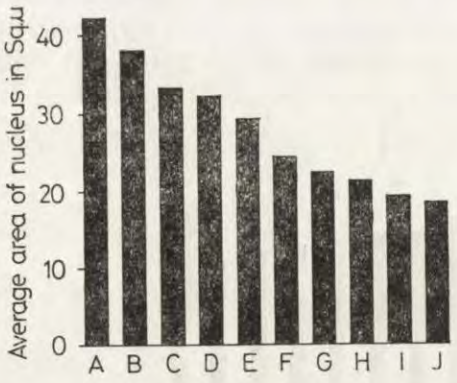

Fig. 4. Histogram showing the average area (in sq. μm) of nucleus of *T. avium bakeri* in different experimentally infected birds. A — common mynah and parakeet, B — red whiskered bulbul, C — pigeon, D — grey headed mynah, E — koel, F — chick and spotted munia, G — red vented bulbul, grey headed mynah and blue jay, H — black breasted quail, I — crimson breasted barbet, J — spotted owllet

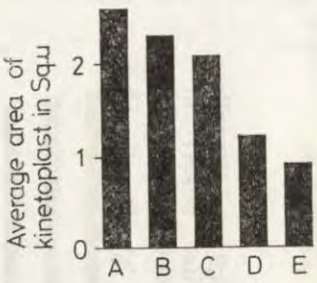

Fig. 5. Histogram showing the average area (in sq. μm) of nucleus of *T. avium bakeri* in different birds (A — from natural infection, B–E — from experimental infection). A — red whiskered bulbul, B — pigeon and common mynah, C — parakeet, spotted owllet and barbet, D — chick, quail, red vented bulbul, pied mynah, spotted munia, koel, domestic duck, little egret and blue jay, E — grey headed mynah

Discussion

The importance of cross-transmission experiments of the trypanosomes has been exhorted by a good number of workers (Baker 1956 a, b, Levine 1961, Bennett 1961, Molyneux 1969).

It has been clearly shown by our transmission experiments that this avian trypanosome has got very little host specificity, so far as the avian hosts are concerned.

Baker (1956 a,c) and Bennett (1961) have experimentally transmitted *T. avium* to different birds. The present observation differs from the earlier ones in many respects. Baker (1956 a,c) has made very limited cross transmission experiments although he has extensively studied the life cycle of *T. avium*. Bennett (1961) also has not incorporated the biological properties and mensural statistics of the trypanosome in different experimentally infected birds. However, Bennett (1970) has suggested that trypanosomes of birds can be identified by their ability to develop in various strains of *A. aegypti*. *T. avium bakeri* has been found to undergo multiplication in *Aedes albopictus* and experimental transmission has been reported by Chatterjee (1977).

In the present investigation, biological properties as well as mensural differences of the trypanosome in different avian hosts have been carefully recorded. It is evident from the histogram (Fig. 2) that the average length and breadth of the body vary considerably in different hosts. Similar mensural differences have been observed by Baker (1956 a) during experimental transmission of *T. avium* and also by Singh et al. (1956).

The biological behaviour of the trypanosome in different experimentally infected birds has also been found to vary to a certain extent. In most of the experimentally infected birds, the flagellates have been found to appear in the peripheral blood within ten to fiften days after inoculation. In some of the birds, the flagellates appear in the peripheral blood even after 18 days. Such variation has been encountered even when equal volumes of citrated blood, containing the flagellates collected from the same pigeon, were inoculated into different species of birds. It has also been seen that the nature and duration of parasitemia are not similar in all experimental hosts. Infection persists for a short time in some birds (e. g., chick, duckling and blue jay) while in other birds (e.g., pigeon and parakeet) infection remains for quite a long time. Parasitaemia in some cases (e.g., chick, duckling, quail, egret and blue jay) has been of low grade. Conversely, high

grade parasitaemia has been noted in some hosts (e.g., pigeon, parakeet, koel, mynahs and bulbul).

Therefore, the susceptibility of birds to this trypanosome definitely varies. In the more susceptible host, the frequency of multiplication of the parasite is quite high and always different types of developing forms are seen in the bone marrow. Since it is an experimental study with a single species of trypanosome, it is believed that such variation and differences as reported above, might be due to the influence of the physiology of the host on the parasite.

The birds from different orders of classification have been taken mainly to see if any bird is resistant to the infection. The taxonomic situation of avian trypanosome is definitely not clear. Baker (1975) made a very justified attempt in resolving the problem. According to Baker (1975) this trypanosome should be treated as a sub-species of *T. brimonti* Mathis and Leger, 1910, and not as a sub-species of *T. avium*.

However, the ability of this trypanosome to infect spotted owl under experimental infection has created some confusion. Baker in a personal communication suggested that future work on the natural infection of *T. paddae* might help in solving the problem.

The review of the results of the cross transmission experiments of the present work suggests that some mensural and biological variations might arise in the same species of trypanosome in the different experimentally infected avian hosts. In the present state of knowledge when it is seen that avian trypanosome possesses very little host specificity, it may be proposed to consider *Trypanosome avium* as a polytypic species as has been proposed by Davis (1952) for lewisi-like trypanosomes.

Thus, until there is clear evidence to the contrary, I feel that this trypanosome fits more into the sub-species of *T. avium*.

ACKNOWLEDGEMENT

The present work was carried out in the Presidency College, Calcutta and the author is very grateful to Prof. S. Mookerjee and Late Prof. H. N. Ray for their valuable suggestions and endless encouagement.

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ACTA PROTOZOOLOGICA
Vol. 22, No. 1, pp. 55–70 (1983)

Studies on the Biology and Cytology of *Parastasia fennica* (Michajłow) (*Flagellata, Euglenida*), a Parasite of the Intestine of *Cyclopidae* (*Copepoda*)

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Received on 2 July 1982

Synopsis. The developmental cycle of *Parastasia fennica* (Michajłow) have been studied, using morphological and cytochemical methods. Trophozoites, their adaptations to parasitism, palintomic divisions and flagellate forms are described in detail, and the differences between the studied populations and those from Finland (Michajłow 1966) are noted. Cytochemical investigations have shown that stored food substances in *P. fennica* are: paramylon, a polysaccharide of the glycogen type, and acid mucopolysaccharides, besides, great amounts of RNA have been found in the cells. Nucleus of *P. fennica* — as opposed to other euglenoids — does not contain central nucleolus. Numerous mitochondria are distributed peripherally and near the nucleus. In the pellicle of individuals emerging from the host's intestine acid mucopolysacharides have been detected, while flagellate post-palintomic forms contain but little reserve nutriment. Finally, the nature of parasitism in *P. fennica* is discussed, with special emphasis laid on its obligatority.

Parastasia fennica (Michajłow 1966) was originally found in the intestine of *Acanthocyclops viridis* (Jur.), *Eucyclops macruroides* (Lill.) and *Macrocyclops fuscus* (Jur.) (*Copepoda: Cyclopidae*), collected in the littoral zone of lakes, as well as in small water bodies, in the vicinity of Helsinki, it has been described by Michajłow (1966, 1972).

The developmental cycle of *P. fennica* — like that of the remaining species of the genus — consists of two distinct phases: (1) the trophic phase, associated with parasitizing in the host's intestine, and (2) the reproductive phase, while the protist lives in the external

environment, i.e., in water. This species reproduces by means of several consecutive palintomic divisions, and the resulting flagellate forms get into the cyclopid's intestine with food (Michajłow 1966, 1968, 1978).

Electron microscopic studies by Fize and Michel (1972) have contributed to determine the systematic position of the present species, showing its ultrastructural similarity to representatives of the genus *Astasia*.

Prior to cytochemical study of geographically different populations of the species, it was necessary to establish the range of its hosts, as well as to investigate its developmental cycle with special attention to details of morphology and functional activity of all the stages involved, morphological and morphometric methods were applied together with observation of living protists.

Material and Methods

Copepods were collected in small lakes and ponds in the vicinity of Leningrad during the summers of 1979 and 1980. In mid July of 1980 small water bodies yielding plankton dried up completely, so, to continue our studies, we cut out ca. 10 cm thick pieces of soil, put them into an aquarium, and pour settled tap-water over them, within 5–6 h copepods appeared, sometimes harbouring individuals of *Parastasia* in the intestine. The prevalence and intensity of infestation has been determined immediately after collection of the material.

Some copepods were left in small aquaria for further investigation. More than 100 individual cultures (Michajłow 1964, 1966) of infested hosts, and then of the parasites emerging from their intestine, were carried on in the room temperature (20–25°), in a drop of water placed in humid chamber on the glass slides. These cultures, constantly controlled under microscope, enabled us to observe the successive changes undergone by trophozoites during developmental cycle, as well as to study stages of their division and flagellate forms.

The identification of the species and description of its developmental cycle have been based on both living an fixed material. The fixatives used were 4% and 10% neutral formalin and Carnoy's fluid. Mayer's acid hemalaun with eosin and azure-eosin after Giemsa-Romanovsky served to stain the flagellates for general morphology. The nucleus was stained according to the Feulgen reaction, while the methyl green-pyronin method after Unna, and the gallocyanin-chromalum

method after Einarson, were used for study DNA and RNA; RNA was also examined on the preparations treated with 2% solution of toluidine blue after Brachet, control specimens being exposed to ribonuclease (Pearse 1968). Sudan black B was employed for detection of phospholipids, and red sudan III — for neutral lipids, in both cases saturated solutions in 70% ethyl alcohol were used. Control preparations were treated with 1 : 1 mixture of chloroform and acetone. Polysaccharides were revealed with Lugol's iodine and PAS-reaction after McManus and Hotchkiss amylase digestion served as control), while staining with 0.1% solution of alcian blue in 3% acetic acid after Steedman, and with 0.1% toluidine blue in 30% ethyl alcohol, made the determination of mucopolysaccharides possible (in this case hyaluronidase served for control).

Mitochondria were revealed by dyeing with light green aqueous solution according to Novelli's (1959) method, and the protein components of both cytoplasm and nucleus — by treating with bromphenol blue solution in sublimate. Finally, staining cells *in vivo* with 0.01% solution of alcian blue (Krylenkov et al. 1979) made them suitable for observations of the pellicle and of the sheath formed a round dividing individuals.

Results

Hosts, Prevalence and Intensity of Infestation

The parasite under study was found in the intestine of 7 species of *Cyclopidae: Acanthocyclops bicuspidatus* (Claus), *Acanthocyclops viridis* (Jur.), *Acanthocyclops vernalis* (Fisch.), *Acanthocyclops bisetosus* (Rehb.), *Mesocyclops leuckarti* Claus, *Macrocyclops fuscus* (Jur.), and *Eucyclops serrulatus* (Fisch.). In most cases the host's intestine contained 1–2 individuals of *P. fennica*, but fairly frequently they were 3–4 or more, occasionally even 20–30, and one of the copepods harboured as many as 35 parasites. Usually big individuals of *Parastasia* occurred singly or by few, instead, where they were more than ten, they remained small until they left their host. Furthemore, the phenomenon of successive maturation and emerging from the host's intestine have been observed. The prevalence of infestation varied among particular water bodies and periods of study, ranging from 1–2% to 80–90%.

Trophic Phase of the Developmental Cycle

Morphology of Mature Trophozoite

The species characters are the best manifested in mature individuals trophozoites, parasitizing mainly in the middle portion of the host's intestine (Fig. 1 a, 2). The body of the trophozoite is elongated, cylin-

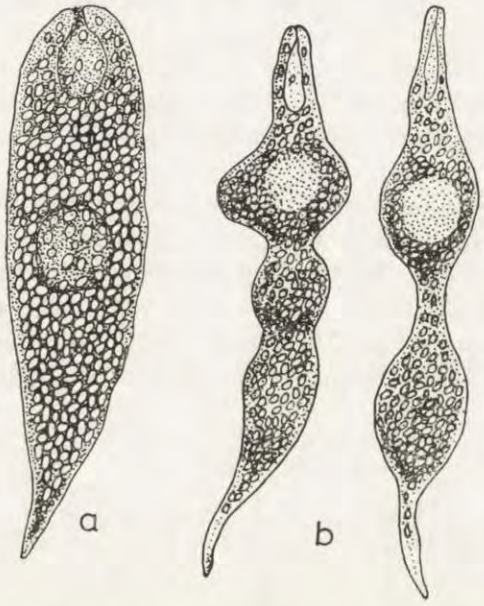

Fig. 1. Mature parasitic form of *Parastasia fennica*, a — morphology of a cell taken from the host's organism, b — individuals moving metabolically in water

drical in shape, round in cross-section. Mature individuals, ready to leave the host's intestine, attain 200–210 μm in length and 40–50 μm in width at mid-length. Before being expelled from the host gut, the parasite ceases moving and turns spherical. Having left the intestine the flagellate remains immobile for a short while, and then resumes metabolic movements (Fig. 1b).

In the anterior part of the body there is an opening (cytostome) leading to the canal (gullet) which expands into an oval reservoir up to 15 μm in length and 10–12 μm in width. It is especially easy to observe when the trophozoite moves, the anterior part of its body stretches out, and the shape of the reservoir changes considerably. Inside of the reservoir there is a thin, slightly arcuated flagellum emerging from its bottom and attaining 10–15 μm in length. Hardly visible *in vivo*, it performs slow oscillatory movements, becoming motionless for few seconds from time to time.

Contractile vacuole is seen beside the lower part of the reservoir. It arises as the result of fusion of small vacuoles formed in nearby cytoplasm into one big, and further swelling, entity. When formed and swollen, the vacuole is separated from the reservoir by a very thin layer of cytoplasm, which disappears from time to time — making a common cavity with the reservoir (emptying vacuole does not simply throw out its contain into the reservoir, instead it seems to join with the reservoir cavity, becoming a part of it) — and is then rebuilt. Swollen vacuoles are formed periodically at regular intervals of few seconds.

The tapering posterior end of the trophozoite body is usually transparent, without distinct granulations.

The body surface of living, as well as fixed and stained trophozoites, as observed under the light microscope, is smooth, compact, underlain by ca. 3 μm thick layer of the ectoplasm. Dark, grey endoplasm contains numerous (several hundreds in mature individuals) grains of a carbohydrate, analogical in size and shape to the paramylon described in *Astasia* and *Euglena:* in *P. fennica* they are oval, 1–3 μm long.

A spherical nucleus, 25–30 μm in the diametr, is located near the center of the cell. It is easily seen, being surrounded by a zone of clear cytoplasm with relatively scarce paramylon grains.

In order to elucidate some particulars of the cell structure in *P. fennica,* several chromatic cytochemical reactions were conducted. Numerous droplets, and diffusive, peculiar orange-red colouration of all the cytoplasm of cells treated with red sudan III, point to a large amount of neutral lipids (Fig. 3). Similarly, sudan black B — serving to reveal phospholipids — dyes the cytoplasm diffusively dark grey, in this case lipid droplets are not seen, but the pellicle and the wall of the reservoir become intensely coloured (Fig. 4)

Stained with bromphenol blue in sublimate solution, all plasmatic structures (with the only exception of paramylon grains) take on various shades of blue, demonstrating protein components of the cell, however, stored protein reserves in form of cytoplasmic grains have not been found in mature trophozoites.

For the study of polysaccharides several cytochemical methods were applied. When total preparations of mature trophozoites were treated with Lugol's iodine the paramylon — the most essential polysaccharide in *Parastasia* — remained colourless, whereas small (ca. 1 μm) grains became brown, and the cytoplasm took on a light-brown diffusive tinge.

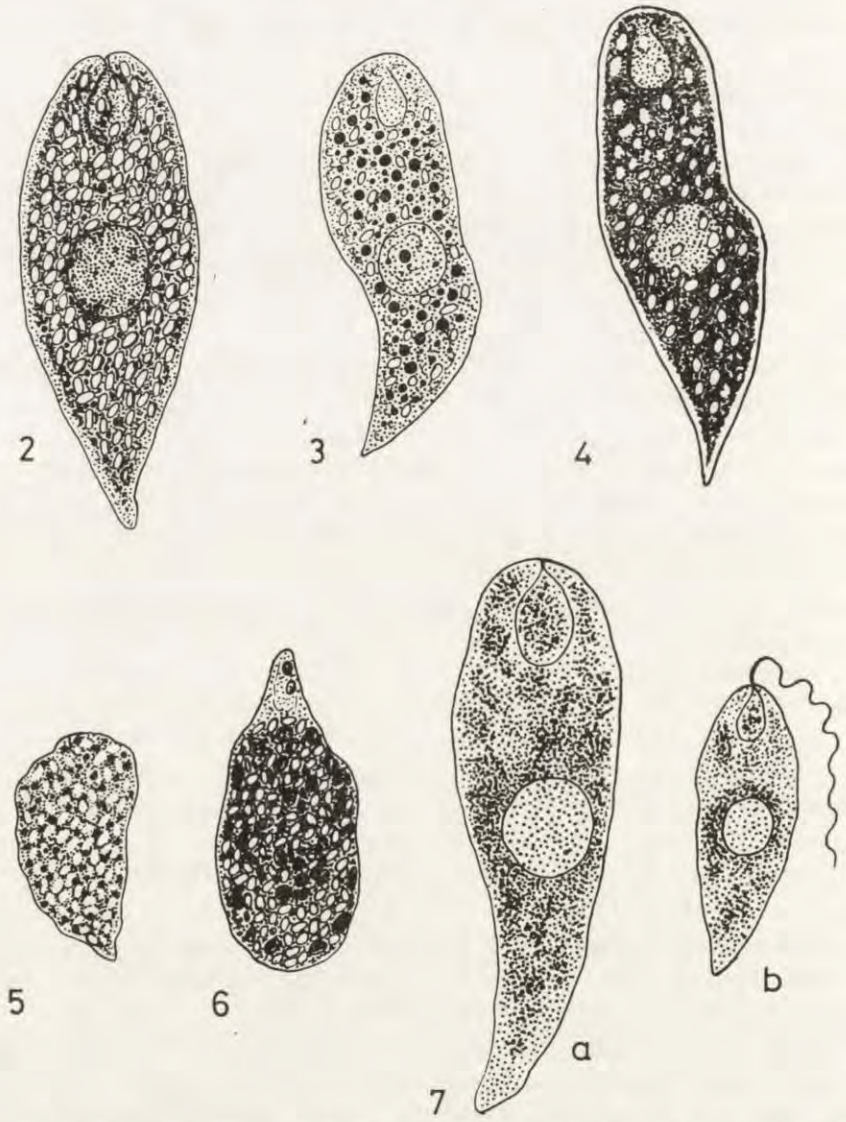

Fig. 2–7. Cytochemical reactions executed on *P. fennica*, 2 — trophozoite staned with acid hemalaun after Mayer, 3 — trophozoite stained with red sudan III, 4 — trophozoite stained with sudan black B, 5 — trophozoite exposed to the PAS-reaction, 6 — trophozoite treated with toluidine blue solution, 7 — trophozoite stained with methyl green pyronin, 7 — flagellate form stained with methyl green pyronin

The PAS-reaction yielded similar results: positively reacted small (ca. 1 μm) grains (but not those of paramylon), distributed over various parts of the cell (Fig. 5), and the cytoplasm assumed distinctive reddish violet colouration characteristic of the glycogen type polysacchari-

des. The control slides, treated with amylase, proved PAS-negative, supposedly the cell of *P. fennica* contains some amount of polysaccharides of the glycogen type.

Toluidine blue, 0.1% solution in 30% ethyl alcohol, stained some polysaccharide (but still not paramylon) grains intensively red (γ-metachromasia) and violet (β-metachromasia) (Fig. 6), the same group of grains proved stainable with alcian blue — both reactions are characteristic of acid mucopolysaccharides — whereas treatment with hyaluronidase made the control specimens react negatively to both, so it seems evident, that the mature trophozoites contain acid mucopolysaccharides as stored and structural substances.

Comparison of the results of all the cytochemical reactions mentioned above suggests, that there are several kinds of stored food substances in *P. fennica*, and that paramylon, a polysaccharide of the glycogen type, and acid mucopolysaccharides, are to be numbered among them. The fact, that the cytoplasm of the mature trophozoite is filled with polysaccharide grains — the bulk of them being the paramylon — shows undoubtedly great importance of these substances as the main source of energy as well for the trophozoite itself, as for the later generations originating from its divisions during the reproductive phase of the developmental cycle.

Strong staining with pyronin following the Brachet's method (Fig.7), as well as with gallocyanin, proves that the mature trophozite contains much RNA in its cytoplasm, what may serve as an evidence of great intensity of the processes of synthesis — especially protein synthesis — in the cell.

Dyeing with acid fuchsin makes mitochondria clearly visible, they are numerous all over the cell, rod-shaped, 1.0–1.4 μm long.

Spherical nucleus is located at the middle of the body. Staining with acid hemalaun after Mayer, or with azur-eozin after Romanovsky–Giemsa, makes fine-grained structures of interphase nucleus apparent (Fig. 2). The Feulgen reaction shows small conglomerations of DNA dispersed all-over the nucleus. One of the distinctive features of *P. fennica*, as compared with the other *Euglenida*, is the lack of compact, centrally situated nucleolus, in our species it is of the diffusive nature, as it can be assumed on the grounds of the results of staining with methyl green pyronin after Brachet: the nucleus takes on homogeneously (without discernible conglomerations of chromatin) — (Fig. 7) pink tinge, characteristic of RNA. The nuclei of mature trophozoites remain at interphase — no division takes place within the host's organism.

Growing Trophozoites

Frequently seen in the cyclops intestine are young trophozoites, whose size depends upon how long they lived there, the smallest individuals were 30–35 μm long and 20–25 μm wide. Morphologically they are similar to the mature trophozoites, but their cytoplasm is clear, containing small of stored food reserves: polysaccharides and lipids (Fig. 9). They feed intensely on the host's food, and their cytoplasm grows darker as reserve nutriments are being accumulated. Those of more than 100–120 μm of length have already considerable resources of lipids and polysaccharides, such individuals — even though their development in the cyclops have not been completed — are able to live and reproduce in water, while smaller ones die if out of the host. Under laboratory conditions, in aquarium, trophozoites live in the cyclops gut for 7–10 days, during that time they accumulate great amount of stored substances, grow to 200–210 μm, and finally spontaneously leave the host's intestine with its excrements.

Reproductive Stage of the Developmental Cycle

Reproduction

Shortly after emerging from the host's intestine the trophozoite commences to shift on the substratum by metabolic longitudinal contractions of the body (Fig. 1 b) — even in water the trophozoite does not form a free flagellum, retaining only the one in the reservoir. Within ca. 30 min the movements get slower and slower, and eventually the protist fastens with its posterior end to the ground, while its body rounds out to become spherical, of about 100 μm in diameter. In many cases transparent slimy sheath (3–4 μm thick) appears on its surface (Fig. 10 a). This sheath — stainable *in vivo* with alcian blue, what allows to suppose, that it contains acid mucopolysaccharides characteristic of the glycocalyx — is a temporary predivisional feature, and in many individuals is not formed at all.

In individual cultures dividing flagellates may be seen 12–14 h after leaving the host's intestine (Fig. 10 b). The division runs longitudinally, and the daughter individuals frequently remain attached to one another at their posterior ends. The second division results in appearance of four (Fig. 10 c), and the third one — of eight (Fig. 10 d) descendant cells, which either remain together or disperse. Quickly after the third division follows the fourth, fifth, and sixth, giving rise to 16, 32, and

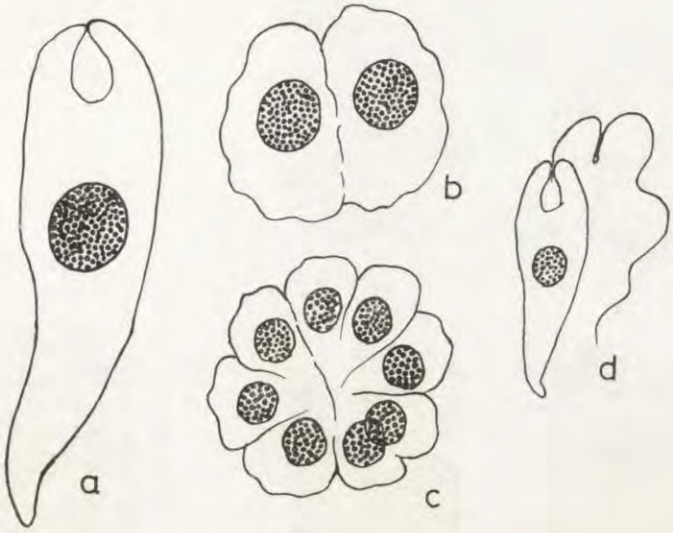

Fig. 8. Stages of development of *P. fennica* (Feulgen reaction) a — trophic form, b — trophic form at the first palintomic division, c — trophic form at the third palintomic division, d — flagellate forms

finally 64 daughter protists (Figs. 10 e, f, g), if they, instead of scattering, form a compact group of cells, then — as the figures show — this stage reminds the metazoan morula.

Palintomic divisions in *P. fennica* follow a very typical course. They are longitudinal, proceed quickly, and are not accompanied by growth of the cells, so that descending individuals decrease in size. Each division begins with nuclear fission (the daughter nuclei being decidedly Feulgen-positive),

Fig. 9. Young trophic form of *P. fennica*

and only then cytokinesis takes place (Fig. 8 b, c). In about 12 h the multiplication process comes to its end, originating 64 daughter cells of ca. 20 μm in length and 10–15 μm in width. They disperse by metabolic movements, but immediately a flagellum starts to grow at their anterior end, and when it is fully developed (in ca. 30 min.), it takes a main part in the protist's movements (Fig. 11), the rise of the flagellum — just as the division itself — occurs at different time in different individuals (Fig. 11).

Full sequence of palintomic divisions results usually in arising of 64 flagellate forms (representing the infective stage in the life-cycle of *P. fennica*), but some deviations sometimes occur. For instance, trophozoites of less than 200 μm in length, taken out of the host's intestine,

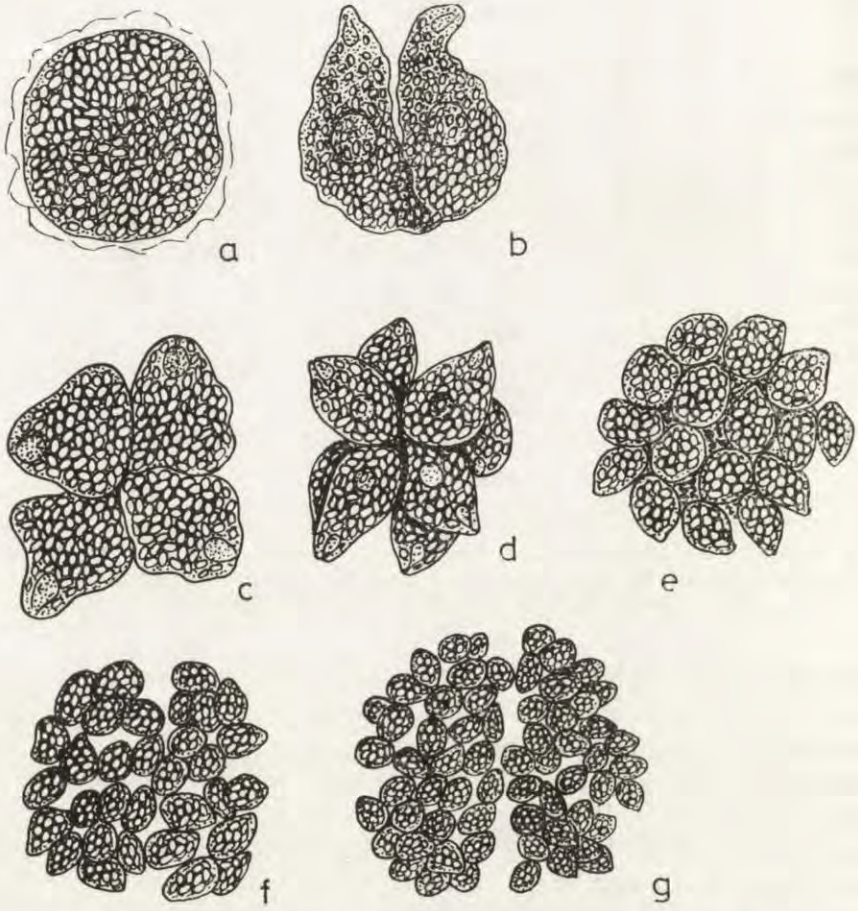

Fig. 10. Palintomic division in *P. fennica*, a — parasitic form before the first division, b — 2 daughter individuals, c — 4 daughter individuals, d — 8 daughter individuals, e — 16 daughter individuals, f — 32 daughter individuals, g — 64 daughter individuals

survive in individual cultures and begin to divide producing, however, only 4, 8, 16, or seldom 32 daughter cells to be transformed into flagellate forms, such individuals are bigger, attaining 35–40 μm in length and 8-10 μm in width (Fig. 12 a). Even the trophozoites of little more than 100 μm are albe to divide (provided they have accumulated enough food reserves), but the number of resulting flagellate forms depends upon the size of the mother cell; very young trophozoites, less than 100 μm, usually die in culture when removed from the cyclops intestine, supposedly — besides other factors regulating divisions — because their reserves of stored nutriment (paramylon), the source of energy for mul-

Fig. 11. Formation of flagellate forms on terminating palintomic divisions (64 daughter individuals)

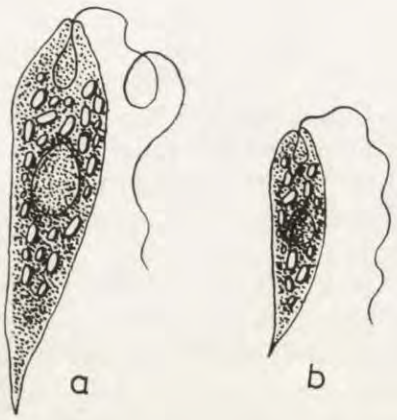

Fig. 12. Flagellate forms of *P. fennica*, a — 40 μm long individual, b — 20 μm long individual

tiplication, is insufficient. Sometimes even fully mature trophozoites of about 200 μm go through but incomplete set of divisions, and produce merely 32 flagellate forms of 40 μm in length.

Flagellate Forms

Flagellate forms constitute the free-swimming stage in the developmental-cycle of *P. fennica*, adapted to living for a short time in water. Irrespective of the differences in size — depending on the number of divisions of the mother cell — the morphological features of flagellate forms are alike and typical of the species (Fig. 12). They are elongated, fusiform, with rounded anterior end of the body, where ovale reservoir is clearly seen. A long, thin flagellum extends out through the opening of the reservoir, its length is variable, being either equal to, or 1.5–2 times greater than the length of the body. Due to the flagellum the protist swims swiftly in water. Flagellate forms frequently attach themselves to the substratum with their posterior end, in sedentary forms the flagellum moves constantly, attracting cyclops.

The body surface of the flagellate forms, as seen under light microscope, is smooth. Thickness of the ectoplasm layer is about 1 μm. Endoplasm is clear, containing not numerous grains of polysaccharides, represented mainly by paramylon, as shown in the Fig. 12, flagellate forms retain several tens of paramylon grains. Negative results of PAS-

reaction proves, that there are but small amounts of acid mucopolysaccharides.

As compared with mature trophozoites, the resources of reserve polysaccharides in flagellate forms are rather scarce and decrease gradually: towards the end of the life span in water (after 3–4 days) the cytoplasm contains merely 2–3 grains of paramylon and becomes transparent. Flagellate forms retain very small amount of lipids, too.

Staining with gallocyanin and methyl green pyronin allows to conclude, that the cytoplasm of flagellate form contains considerable quantity of RNA, but the colouration is much fainter than, in mature trophozoites (Fig. 7b).

In both small and big flagellate forms the nucleus is situated at the middle of the body. It is easily seen *in vivo*, in stained preparations its structure is clearly visible. It remains at interphase (the flagellate forms never divide) and attains 6.5–7.0 μm (small individuals) to 8.0–9.0 μm (bigger ones) in the diameter. Feulgen reaction gives a decidedly violet colouration to the nucleus, showing fine-grained structure of DNA all-over its volume (Fig. 8d), while the cytoplasm and nuclear membrane remain (as in trophozoites) colourless. Methyl green-pyronin stains the nucleus green with pink hue, due to diffusive RNA (after treatment with ribonuclease the pink cast disappears).

As in mature trophozoites, the nucleolus is impossible to be detected under light microscope.

Feeding on microplanktonic and microbenthonic organisms, cyclops swallow flagellate forms of *P. fennica*, which lose their flagella and become young trophozoites (Fig. 9). The flagellates which fail to get into cyclops intestine use all their paramylon resources up and die in 3–4 days.

Discussion

Parastasia fennica is here reported for the first time from the vicinity of Leningrad. The specimens obtained from *Copepoda* collected in water bodies of that area are bigger and show certain morphological, as well as biological, differences in comparison with those described by Michajłow (1966) from Finland, nevertheless, an analysis of morphology and developmental cycle allowed to identify them as representatives of the same species.

The maximum prevalence (up to 90%) have been stated in three species of cyclops: *Eucyclops serrulatus* (Fisch.), *Acanthocyclops viridis* (Jur.), and *Macrocyclops fuscus* (Jur.). In those water bodies, where all

the three occurred, *M. fuscus* (being also in Finland one of the main hosts of *P. fennica*) showed the highest prevalence infestation, in its absence *E. serrulatus* was the most frequently infested. The host range of *P. fennica* is rather limited, as it has already been noticed by Michajłow (1966).

No essential distinction in the morphology of trophozoites parasitizing in different species of cyclops have been stated.

The developmental-cycle of the species under study is presented on the diagram (Fig. 13), being a summary of what has been reported in

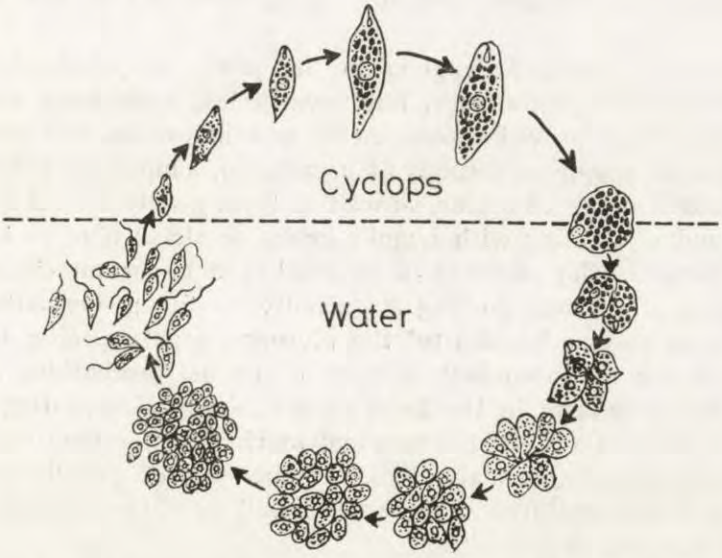

Fig. 13. Diagram of the developmental-cycle of *P. fennica*

the preceding chapters. Under laboratory conditions, the trophic phase in the host's intestine lasts 7–10 days, and the reproductive phase in the external environment — including the lifetime of flagellate forms — extends to about as long.

Contrary to the free-living species of *Euglena* and *Astasia*, *P. fennica* has no nucleolus appreciable under light microscope, the nucleolar substance being diffused all-over the nucleus. The cytoplasm of the present species contains much RNA, especially in the perinuclear zone. what suggests its intense production in the nucleus and vigorous penetration from there to cytoplasm. Indeed, a study with electron microscope showed great number of ribosomes in cytoplasm, most of all near the nucleus (Fize and Michel 1972).

P. fennica is a heterotrophic organism, showing high degree of specialization and adaptation to endoparasitic life in the cyclops' intestine. Elongated, fusiform shape of trophozoites and their faculty to move metabolically are features enabling them to remain in the host's intestine until maturity. Trophozoite is the form accumulating food reserves, quick growth, as well as increasing amount of stored polysaccharides and lipids, are indicatives of the intensity of its feeding. Food vacuoles containing solid nutritive substances have not been found in the body of trophozoites — they feed probably by means of pinocytosis, taking liquid or semiliquid nutrients digested by host's enzymes, indeed pinocytotic vesicles are seen on electron micrographs (Fize and Michel 1972).

Cytochemical methods applied to the study of polysaccharides in *P. fennica* yielded preliminary, but nevertheless interesting data about their nature. They proved heterogeneous in composition. The majority of polysaccharide resources consists of paramylon, similar — judging from the size and features of grains, as well as from negative results of PAS-reaction and of staining with Lugol's iodine or alcian blue — to that of other *Euglenida*. The presence of paramylon is important characteristic of the present species, proving its affinity to the genus *Astasia*. The PAS-positive polysaccharides (of the glycogen type), specific to animal cells, are much less abundant. A part of the polysaccharides occurring all-over the cytoplasm in the form of grains are — according to their cytochemical reactions — acid mucopolysaccharides, serving not only as stored food reserves, but also being components of pellicle and other membranaceous structures of the cell, as well as of the ephemeral slimy sheath of dividing trophozoites.

In the course of developmental-cycle the amount of stored polysaccharides — especially paramylon — undergo considerable changes. Because of unapplicability of biochemical methods for study of polysaccharides in *P. fennica*, quantitative variations of paramylon can best be visualized by dividing flagellates into groups according to the number of grains in cell, this method is frequently employed by students of these substances *in Protozoa* (Poljanskij 1963, Sukhanowa 1968, Lozina-Lozinskij, Byčenkova 1978). The first group includes trophozoites at earliest stages of development in the host's intestine, containing less than 10 small grains of paramylon (Fig. 14), here belong the flagellate individuals, too. 50–60 μm long trophozoites whose cytoplasm comprises several tens (30–40) of paramylon grains (both small and attaining 3 μm in length) have to be assigned to the second group. The third group consists of trophozoites of up to 100 μm in length, ha-

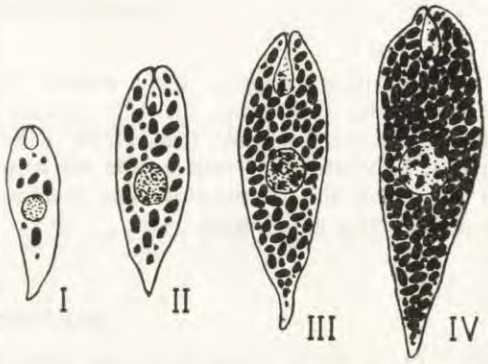

Fig. 14. Classification of the individuals into groups according to the number of paramylon grains

ving accumulated considerable amounts of paramylon: their body is filled by big grains of the polysaccharide. Finally, to the fourth group belong mature, 150–200 μm long trophozoites with big paramylon grains crowding their cytoplasm.

Living in host's intestine the trophozoite grows, and the number of paramylon grains in its body increases, what shifts the ranking of the individual from the group I to IV: mature trophozoite, emerging from the cyclops intestine to water and ready to asexual reproduction, contains paramylon resources of the highest group. Each binary fission results in approximately even segregation of polysaccharides between the daughter cells, however, they become less and less abundant (the ranking shifts back from the group IV to I), as rapid succession of divisions is accomplished at the cost of the energy originating from the enzymatic decomposition of paramylon.

The expenditure of paramylon by dividing trophozoites and flagellate forms shows that after quitting the cyclops organism, throughout its life in water, *P. fennica* does not feed, subsisting exclusively on polysaccharides accumulated during the trophic phase. Enormous amounts of paramylon in mature trophozoite is an adaptations to obligatory parasitism, another characteristic related to such a way of life is great number — 64 in our experiments, but up to 128 according to literature (Michajłow 1966, Fize and Michel 1973) — of flagellate forms produced by palintomic division of each mature trophozoite: by far not all of them find their way to cyclops organism within the short time of their survival in water, many die or are eaten by another animals feeding on microbenthos or microplankton.

Features of adaptation to parasitic life, manifested throughout the developmental-cycle, prove that *P. fennica* is an obligatory parasite of *Cyclopidae*.

ACKNOWLEDGEMENTS

The Authors express their sincere thanks to the Head of the Laboratory of Unicellular Organisms, Institute of Cytology at the USSR Academy of Sciences, Member of the Academy, Prof. Dr. J. I. Poljansky, for making it possible to work in that Laboratory, as well as for his help in carrying out the present study and in preparing the manuscript. The technical assistance of Miss A. I. Radczenko is also greatly appreciated.

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ACTA PROTOZOOLOGICA
Vol. 22, No. 1, pp. 71–77 (1983)

The Rat as an Animal Model of *Giardiasis*

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Received on 28 July 1982

Synopsis. The purpose of the study was to observe the course of giardiasis in Wistar rat spontaneously infected and in *Giardia*-free succeeding generations of rats infected experimentally with *G. muris*. This study shows different course of giardiasis in conventional and *Giardia*-free rats. The rat model of giardiasis induced experimentally, is characterized by long-termed chronic infection, without periods of negative stools and spontaneous elimination of the parasite. It is possible to infect most of the rats by exposure to a very small dose of viable *G. muris* cysts.

Giardiasis is a protozoon infection which recently attracted special attention as parasitologists currently agreed that *G. intestinalis*, the parasite of human upper digestive tract, is a pathogenic protozoon and the most common cause of disease associated with drinking water supplies in the USA (Healy 1979). Nevertheless, many problems remain unsolved, e.g., host specificity of *Giardia* sp., viability and infectivity of parasites cysts and pathologic features of the hosts intestine. To study these problems an animal model would be of great importance. Stevens and Roberts-Thomson with co-workers developed an animal model using *G. muris* in mice to study the role of immunity in the pathogenesis of giardiasis (Stevens et al. 1977, 1978 a–c, Roberts-Thompson et al. 1976, 1978, 1980).

The purpose of the present study was to observe the course of giardiasis in Wistar rat spontaneously infected and in *Giardia*-free succeeding generations of rats infected experimentally.

Materials and Methods

Cyst source. *G. muris* cysts were obtained from fresh feces of four cyst-passing Wistar rats, infected experimentally with the same population of cysts. The species was established as *Giardia muris* on the basis of trophozoite morphology (shape of the cell and relation of the adhesive disk to the cell length, shape and position of median bodies).

Cyst inoculum. *Giardia* cysts were purified by a slightly modified procedure of Bingham et al. (1979). After suspending the feces in tap water and filtering through three layers of gauze, the filtrate was centrifuged several times at 600 g for 5 min to remove small particles. The resuspended sediment (3 to 5 ml) was placed on 3 ml of chilled 0.85 M sucrose solution and centrifuged in 10 ml centrifuge tube at 600 g for 5 min at room temperature in a swinging bucket rotor. The water-sucrose interface containing the cysts was removed, diluted 1 : 10 with water, and recentrifuged for 5 min. To achieve *Giardia* cysts free of fecal matter, sequential sucrose gradient centrifugation of the water-resuspended pellet was repeated. The final pellet was resuspended in water and the cysts were counted in a hemacytometer chamber. The number of viable cysts was calculated by using Bingham and Meyer excystation procedure (1979) and eosin exclusion. The volume of the inoculum was 0.1 ml.

Rats. Gravid outbred female Wistar rats infected with *G. muris* were selected for obtaining the spontaneous infected litters of animals. The first *Giardia*-free generation was bred from rats free of *Giardia* cysts for 20 consecutive days. Further *Giardia*-free generations (2nd to 4th) were inbred by permanent direct examination of feces of all animals and microscopic examination of intestinal mucosal scrapings from rats randomly selected. The animals were kept in large polypropylene cages, which were cleaned each day. The cages, as well as the water bottles and bedding were sterilized before use. The cages with *Giardia*-free colonies of rats were maintained in periodically sterilized boxes situated in a separate room to prevent contamination by *Giardia* cysts. For experimental infection 3-week-old weanling rats, weighing 25 to 30 gm, were used. The cysts were inoculated intragastrically. The animals were fed on standard pellet diet and water *ad lib.* Rats infected spontaneously were kept in groups in the same cages or were isolated in individual cages. All the rats infected experimentally were maintained in individual cages.

Uninoculated rats served as a control group. Microscopic control of freshly excreted feces rats were performed daily; in the case of negative results feces were controlled for 20 consecutive days. Cysts density was designated as +, ++, and +++ (few cysts in whole preparation, 1 to 5 cysts per microscopic field at 20× objective and over 5 cysts per microscopic field at 20× objective, respectively).

Results

The observations carried out on spontaneously infected rats are summarized in Table 1. The results indicate that (1) the infection developed in most animals; (2) the infection rate was of low intensity; and (3) the infection of short duration prevailed. Additionally, the infection with *G. muris* was characterized by frequent periods of negative

Table 1

Wistar rats spontaneously infected with *G. muris*

Rats kept in:		Common cages	Individual cages
Number of rats tested		23	15
Number of infected rats		21	8
Intensity of infection			
(% of stool samples tested)	−[1]	78	63
	+	22	37
	++	0	0
	+++	0	0
Short-termed infections (up to 10 days)		14	5
Long-termed infections (over 14 days)		7	3
Reinfections		12	0

[1] Negative for cysts; + few cysts in whole preparation; ++ 1 to 5 cysts per microscopic field at 20 × objective; +++ over 5 cysts per microscopic field.

stools. This course of giardiasis was observed both in animals kept in groups in common cages and in these isolated in individual cages. The only difference concerned the reinfection which occurred entirely in rats kept in groups in the combined cage; thus we may accept that the reinfection was not autoinfection [1].

The course of experimental giardiasis in the first to fourth primarily *Giardia*-free generations of rats is shown in Table 2 and compared with the course of infection in conventional animals. The percentage of viable cysts estimated by using excystation test or eosin exclusion, varied

Table 2

The course of giardiasis in spontaneously infected and in *Giardia*-free Wistar rats infected experimentally

Giardia-free generation of rats:		0[1]	1st	2nd	3rd	4th
Number of rats inoculated		—	25	21	20	33
Number of infected rats		8	12	18	18	10
Intensity of infection						
(% of stool samples tested)	−[2]	63	63	16	25	48
	+	37	32	73	46	33
	++	0	5	11	24	14
	+++	0	0	0	5	5
Short-termed infections (up to 10 days)		5	7	0	0	4
Long-termed infections (over 14 days)		3	5	18	18	6

[1] Spontaneously infected.
[2] See footnote Table 1.

[1] As reinfection we denote a second infection with *G. muris* after 20 days of negative stools.

within 0 to 26 and 78 to 100 respectively. With the exception of experiments No. II–1, III–2, IV–2 and IV–3, the number of viable cysts in inoculum estimated by *in vitro* excystation test was no smaller than 1.0.

In the first generation of primarily *Giardia*-free rats the number of cysts in inoculum (from 50 to 10 000) had no influence on the number of rats infected. These infections were mainly of short duration, and characterized by small number of cysts excreted in feces. However, most of the *Giardia*-free rats of the second and third generations exposed to different doses of *G. muris* cysts (10 to 1000) became infected, and the infection was long-termed. In all animals the infection existed through the whole period of observation; e.g., in some animals, kept particularly for such long-termed observation, the everyday excretion of cysts persisted for over 350 days. Spontaneous resolution of infection was not observed. The intensity of experimental infection of the primarily *Giardia*-free rats was, as a rule, not high, but was growing more intense in the succeeding generations of the animals.

The results of experimental infection of rats of the fourth *Giardia*-free generation call for further explanations (Table 2). The three groups of rats were exposed to very few cysts (5 in experiment No. IV-1) or to cysts of zero viability when tested by *in vitro* excystation procedure (experiments No. IV-2 and IV-3). In the first group no rat exposed to *G. muris* became infected, but the infection occurred in 1/2 of the rats inoculated with "non-viable" cysts in two further experiments (IV-2 and IV-3). The low dosage of viable cysts may explain the short duration and low level of parasites density in the stools of rats infected in experiment No. IV-3.

The control animals — a group of uninfected rats — showed no evidence of *Giardia* both in feces and in intestinal mucosal scrapings.

Discussion

The search for an animal model in giardiasis is patent as this poorly known host-parasite relationship requires thorough studies. The results of experimental infection of animals with human parasite, *G. intestinalis*, were at least controversial. On the one hand, some experimental infection failed (Sharapov and Soloviev 1977) or required particular conditions (Goritskaya and Vrublevskaya 1966). On the other, there are some questionable results arising from the natural presence of *Giardia* in animals used for experimental infection, e.g., rats qualified as "clean" by three-day negative stools in Sehgal's et al.

work (1976). Since some authors questioned the possibility of cross-transmission experiments (Bemrick 1962), studies on giardiasis in natural host are indispensable.

This study shows different course of *G. muris* infection in conventional (spontaneously infected) and in primarily *Giardia*-free Wistar rats. The infection in conventional rats is characterized by spontaneous disappearance as it was observed in earlier works of other authors both in human (Rendtorff 1954) and in animals (Bemrick 1962, Roberts-Thomson et al. 1976). Furthermore, the rats spontaneously infected showed low cyst excretion with frequent periods of negative stools. The comparison of the spontaneously infected rats kept in groups in the combined cages to these isolated in individual cages indicates easy reinfection exclusively in rats kept in groups. Thus, notwithstanding Bemrick's (1962) findings, it appears that autoinfection of rats isolated in individual cages is at least a very rare occurrence, even if the animals are re-exposed to the cysts excreted by themselves. This observation might be explained by the results of Roberts-Thomson et al. (1978) study. The authors found the resistance to re-infection in mice and suggested that the spontaneous resolution of infection was associated with the immune response.

The experimental infection with *G. muris* in *Giardia*-free rats in this study, beginning from the second *Giardia*-free generation, is characterized — contrary to the infection of conventional animals — by long duration. In fact, the infection persisted through the whole period of observation, which in some animals was extended over 350 days. As a rule, the level of parasites density in the stool was not high but was growing in the succeeding *Giardia*-free generations of rats infected experimentally.

Roberts-Thomson et al. (1976) found in their excelent study on giardiasis in mice that large cyst inocula, increasing earlier the antigenic stimulus, lead to earlier elimination of *Giardia*. In this study we did not find any relationship between the dose of cysts (from 10 to 1000) and spontaneous resolution of infection in fact, in the second and third primarily *Giardia*-free generations no spontaneous elimination of *Giardia* was observed.

The results of experiments in which rats were exposed to very few viable cysts (less than 1 cyst (!) per inoculum in experiments No. II-1, III-2, IV-1, IV-2 and IV-3) as estimated by excystation procedure indicate that very small numbers of cysts, even a single cyst, will cause infection with *G. muris* in rats. Rendtorff emphasized the extreme importance of the fact that a single cyst would produce infection in waterborne transmission of giardiasis (1979). On the other hand the

results of our observations contradict the excystation procedure *in vitro* as an objective test for cyst viability determination (Kasprzak and Majewska, in press).

To conclude, one may accept that the rat model of giardiasis, induced experimentally, is characterized by long-termed chronic infection, without periods of negative stools and spontaneous elimination of the parasite. It is possible to infect most of the rats by exposure to a very small dose of *G. muris* cysts. For this purpose *Giardia*-free bred rats should be used.

ACKNOWLEDGEMENTS

This work was supported by a grant from the Food and Drug Administration, PHS, DHEW, Washington, D. C., USA, and also financed partly by a grant from the Parasitological Committee, Polish Academy of Sciences.

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ACTA PROTOZOOLOGICA
Vol. 22, No. 1, pp. 79–85 (1983)

Trypanosoma batrachi Qadri, 1962 and Its Effect on the Biochemical Composition of the Blood of *Clarias batrachus*

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Received on 7 May 1982

Synopsis. The blood of a fresh water fish, *Clarias batrachus* revealed the presence of the flagellated protozoan parasite, *Trypanosoma batrachi* Quadri, 1962, The desriptions of the dimorphic forms of the present species — "stumpy" and "slender" are provided for the first time. Further studies were carried out on the effect of this parasite on some biochemical parameters viz. glucose, cholesterol, protein, acid and alkaline phosphatase of the blood of *C. batrachus*. Glucose, cholesterol and acid phosphatase decreased whereas protein and alkaline phosphatase increased in the infected fishes by various degrees.

The trypanosome from the blood of *Clarias batrachus* conforms with *Trypanosoma batrachi* Qadri, 1962 from the same host. The dimorphic nature of the parasite had been suggested by the author, but a detailed description of the two separate dimorphic forms named as "stumpy" and "slender" is being given for the first time. The effect of these trypanosomes on the biochemical composition of blood of fishes is practically untouched in India. In the present work, the effect of *Trypanosoma batrachi* on the glucose, cholesterol, protein, acid and alkaline phosphatase levels of the blood of *C. batrachus* was studied showing quantitative rise and fall in the various blood parameters.

Material and Methods

Blood was obtained after severing the caudal peduncle of the fishes which were sacrificed in live condition. In order to study the morphology of the parasite, thin blood films were made, fixed in methanol and stained in Leishman

(1 drop of stain + 7 drops of buffer solution) for 40 min and observed under an oil immersion. For biochemical assay, studies were carried out during November–December when infection was at its peak. Fishes of the same size (20–25 cm) and from the same locality were investigated. Determination of the various parameters was made following the standard techniques as given by Oser (1965).

Observations

Morphology of *Trypanosoma batrachi* Qadri, 1962 (Fig. 1 A–F)

24% infection was observed. "Stumpy" and "slender" forms could clearly be distinguished (Table 1).

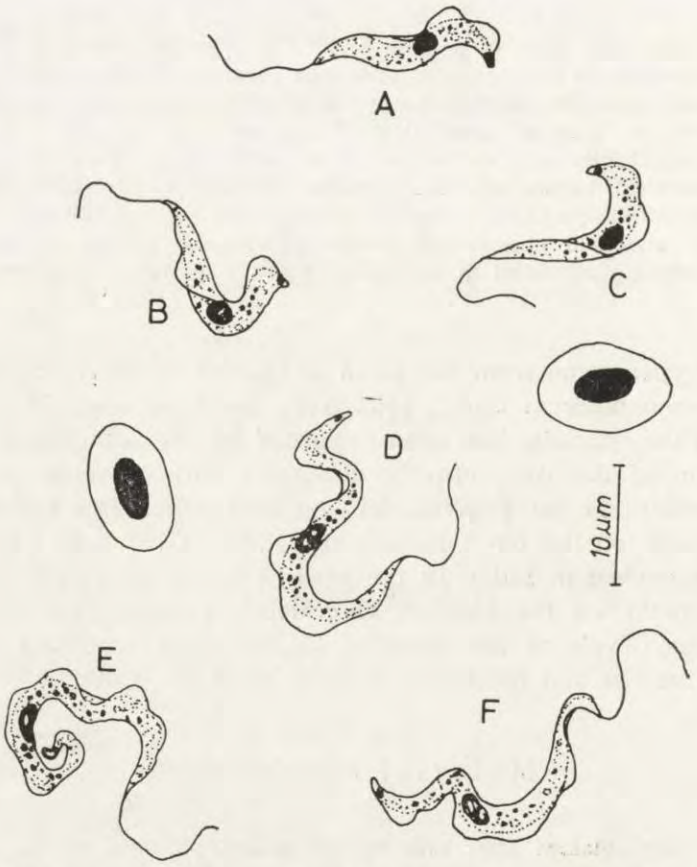

Fig. 1. Camera lucida drawings of *Trypanosoma batrachi* Qadri, 1962. A–C — stumpy forms, D–F — slender forms.

Table 1

Measurements of the various body parts of *Trypanosoma batrachi* Qadri, 1962 (in μm)

Component parts of the parasite	Stumpy form	Slender form
Total length of parasite including free flagellum	28.7 (23.0–33.2)	44.6 (35.6–49.2)
Length of cell body	18.7 (10.2–23.4)	29.4 (26.4–33.2)
Breadth of cell body	2.6 (2.0–3.8)	1.8 (1.0–2.1)
Length of free flagellum	10.0 (7.2–12.7)	15.2 (12.1–18.2)
Length of nucleus	2.2 (1.7–2.8)	2.8 (2.2–3.5)
Breadth of nucleus	2.0 (1.6–2.8)	1.3 (0.7–1.9)
Anterior margin of nucleus to anterior end of body	9.5 (6.2–13.4)	14.8 (10.1–18.2)
Posterior margin of nucleus to kinetoplast	5.5 (3.8–7.8)	9.6 (6.2–14.4)
Length of kinetoplast	0.9 (0.6–1.6)	1.2 (0.9–1.8)
Breadth of kinetoplast	1.0 (0.8–1.7)	0.8 (0.6–1.2)
Distance from kinetoplast to posterior tip	0.6 (0.2–1.2)	1.0 (0.4–1.8)
Width of undulating membrane	0.6 (0.3–1.0)	1.6 (1.0–1.9)

Figures in parentheses give the ranges.

Shape: The stumpy forms are less sinuous, but the slender forms have a sinuous and "S" shaped body. Both the forms have pointed extremities, the anterior end being more acute than the posterior.

Cytoplasm: The cytoplasm is granular in nature, the granules are found on both sides of the body and are more concentrated near the nucleus. Granulation pattern is similar in both forms. Vacuoles of varying sizes are present throughout the cytoplasm.

Nucleus: Topographically, the nucleus is situated behind the middle of the body and is oval in shape. The nucleus of the stumpy form takes a deeper stain and the chromatin granules are more densely packed together. Karyosome is not observed.

Kinetoplast: The well developed kinetoplast is subterminal in position, oval or spherical in shape and broader in size in the stumpy forms as compared to the slender ones.

Blepharoplast: The blepharoplast is absent, the axoneme apparently arises directly from the kinetoplast.

Flagellum: The flagellum originates from the kinetoplast, borders the undulating membrane making 5–8 convolutions and exists beyond the anterior end of the body as a free flagellum. The latter is more developed in the slender forms.

Undulating Membrane: The undulating membrane has only 3–5 folds in the stumpy forms whereas the number increases to 5–8 in the slender forms. The folds are of unequal length.

Measurements: Range and mean values of the stumpy and slender forms are presented in Table 1.

Type host: *Clarias batrachus* (Linn.)
Locality: Ponds in and around Aligarh, Uttar Pradesh, India.
Site of infection: Blood

Effect of *Trypanosoma batrachi* Qadri, 1962 on the Biochemical Composition of the Blood of *Clarias batrachus*

Table 2 shows that a fall in the levels of glucose, cholesterol and acid phosphatase was observed, whereas a rise in the protein and alkaline phosphatase content was observed in the infected fishes as compared to the healthy ones.

Table 2

Effect of *Trypanosoma batrachi* Qadri, 1962 on the blood constituents of *Clarias batrachus* (All values are mean ± SE)

Biochemical constituents	Healthy fish	Infected fish
Glucose (mg/100 g)	65.3±2.2	41.2±3.21
Cholesterol (mg/100 g)	402.1±14.31	354.7±11.80
Protein (gm/100 g)	4.63±0.12	5.31±0.21
Alkaline phosphatase (KA units/100 ml)	5.7±0.20	6.2±0.41
Acid phosphatase (KA units/100 ml)	2.8±0.41	2.4±0.51

Discussion

From India, three species of trypanosomes, *Trypanosoma batrachi* Qadri, 1962; *T. maguri* Tandon and Joshi, 1973 and *T. gadrii* Narasimhamurti and Saratchandra, 1980 have been described from the blood of *Clarias batrachus*. Qadri (1962) reported dimorphism, but did not provide names and mensural data of the dimorphic forms. Tandon and Joshi (1973) mentioned polymorphism in the species described

by them, but did not describe the polymorphic forms separately. Narasimhamurti and Saratchandra (1980) reported monomorphism in *T. qadrii*.

The species under discussion is comparable to *T. batrachi* in the dimorphic nature of the two species and structure and dimensions of the parasite. In the present work, descriptions and mensural data of the two different forms which have been named "stumpy" and "slender" are being given. They had not been named so far. The present species is in conformity with the species mentioned and has thus been identified as *Trypanosoma batrachi* Qadri, 1962, with measurements of "stumpy" and "slender" forms which are being provided for the first time.

The biochemical studies showed that maximum loss was observed in the glucose level ($P < 0.001$), whereas cholesterol declined to the maximum degree ($P < 0.01$) by about 13%. Protein on the other hand showed an insignificant rise ($P > 0.01$). Statistical analysis revealed that the differences in the enzymatic activities of healthy and infected fishes were also insignificant ($P > 0.01$).

Tandon and Joshi (1974) observed that the fishes infected with trypanosomes in blood of some teleosts caused a significant decrease in the blood glucose level. Results of Smirnova (1970) also fall on the same line. Further, it has been established that the presence of pathogenic trypanosomes among higher vertebrates cause a considerable fall in the blood sugar and may even sometimes lead to the death of the animal due to the condition known as glycopyruvic intoxication (von Brand 1973). It may be possible that the pathogenic trypanosomes consume so much sugar in the blood stream that they cause exhaustion of the carbohydrate reserves of the host. As a result, the level of glucose declines significantly ($P < 0.001$) in the fish blood. This view is also in agreement with Schern (1925).

Blood cholesterol level was found to be normal (Linton 1930), elevated (Ada and Fulton 1948) and decreased (Lippi and Sebastiani 1958) during trypanosomiasis, but all these studies were confined to higher vertebrates, specially mammals. The present findings are in agreement with Lippi and Sebastiani (1958). The physiological basis for such changes is unknown, as has already been stated by von Brand (1973).

During the infection with trypanosomes, a rise in protein level of blood is observed in higher vertebrates in which the levels of globulins, specially the γ-globulin rises. Cornille and Hornung (1968) stated that man infected with *T. rhodesiense* may have very high IgM and IgG levels (fractions of immunoglobulins) despite showing approxi-

mately normal γ-globulin values. The same fact may also be true in fishes, where certain fractions of protein elevate while other fractions remain normal causing an insignificant ($P > 0.01$) rise in protein level in the blood of fishes.

Enzymatic activities of fish blood showed a very interesting pattern. Present observations show that alkaline phosphatase activity increased in the infected fishes. Chatterjee and Sengupta (1959) observed that alkaline phosphatase activity increased in hamsters infected with protozoans. Sanchez and Dusanic (1968) also observed that *T. lewisi* infected rats had a high quantity of alkaline phosphatase. It is reported that alkaline phosphatase activity is associated with metabolism of carbohydrates, resorption of sugar, fatty acids (Roche 1950) and probably these may be the main causes of its elevation, because at that time most of the sugar is consumed by parasites and the liver is mostly engaged to synthesize carbohydrates, causing elevation of alkaline phosphatase. Acid phosphatase decreases due to the fact that it replaces alkaline phosphatase in order to initiate the formation of glucose, protein etc.

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ACTA PROTOZOOLOGICA
Vol. 22, No. 1, pp. 87–94 (1983)

On A New Septate Gregarine, *Stenoductus polydesmi* sp. n. (*Sporozoa : Cephalina*) from the Millipede, *Polydesmus* sp. with Observations on Host-specificity of Gregarines Infecting the Millipedes in Kerala, India

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Received on 16 August 1982

Synopsis. A new species of cephaline gregarine, *Stenoductus polydesmi* is described from the gut of the millipede, *Polydesmus* sp., collected from the campus of Sree Narayana College, Cannanore. The gregarine has a ratio of PL : TL = 1 : 7 and PW : DW = 1 : 2.1.

Host-specificity of the gregarines infecting the millipedes in Kerala has been studied. Among the studied gregarines host-specificity ranges from narrow to wide; it seems to depend on specific physiological and biochemical factors obtainable in the midguts of the specific millipede-hosts.

We came across a species of *Stenoductus* in the intestine of an Indian species of *Polydesmus* millipede collected from the campus of the Sree Narayana College, Cannanore, Kerala, in the course of a parasitological survey. Trophozoites and sporonts occur in the millipede's midgut, gametocysts are in the hindgut and/or in fecal pellets. Structural and life-cycle details of the gregarines convinced us that the present form is a new species, unlike those hitherto reported. We propose the name *Stenoductus polydesmi* sp. n. for the present gregarine from *Polydesmus* sp.

Materials and Methods

Taxonomy and Biology: *Polydesmus* sp. collected from the campus of Sree Narayana College, Cannanore, were at once dissected, and the gregarines (trophozoites and sporonts) and their gametocysts recovered were studied follow-

ing the procedure reported by Ramachandran (1976). Figures were drawn with the aid of a camera lucida. The descriptions are based on measurements of a minimum of 20 mature specimens. Abbreviations used in this paper are, DL — deutomerite length, DW — deutomerite width; PL — protomerite length; PW — protomerite width, and TL — total length. The ratios used are, the protomerite length to total length (PL : TL), and the protomerite width to deutomerite width (PW : DW).

Host-specificity: Host-specificity was studied by attempting to release sporozoites of mature spores by simultaneously exposing them to midgut fluids of natural and unnatural sympatric millipede hosts. The spores used were those of *Stenoductus carlogoni*, *S. trigoniuli*, *S. chondromorphi* and *S. polydesmi*, and the midgut fluids were that of *Carlogonus palmatus*, *Trigoniulus goesi*, *Chondromorpha kelaarti*, *Phyllogonostreptus negotiosus* and *Polydesmus* sp.

Results

Stenoductus polydesmi sp. n.

Description

Sporonts (Fig. 1 *1*): Sporonts solitary, white, cylindrical; protomerite hemispherical; deutomerite cylindrical with a broadly round caudal end; constriction between protomerite and deutomerite distinct. Protomerite papilla absent; apical pore absent; epicyte covering of protomerite longitudinally striated, striations continuing onto the epicyte striations of deutomerite. Ectoplasm clear, endoplasm granular, granules measured 1.4 μm to 2.4 μm, staining metachromatically with toluidine blue. Septum circular, flat or slightly convex toward deutomerite, 1.4 μm thick.

Deutomerite cylindrical (rarely slightly dilated at the anterior one third) ending in a broadly round caudal end. Epicyte hyaline, uniformly thick, longitudinally striated; endoplasm granular.

Nucleus spherical to slightly ovoid, feebly visible in fresh sporonts, variable in position, often centrally located in deutomerite. Endosome round, with a round body inside; position of endosome variable inside the nucleus, deep-staining with hematoxylin; extraendosomal region of nucleus clear. Nucleus in a sporont of 128.7 μm by 42.6 μm measured 18 μm; its round endosome was 6 μm in diameter.

Measurements (in micrometers): Measurements of sporonts (with mean in parenthesis) are noted below:

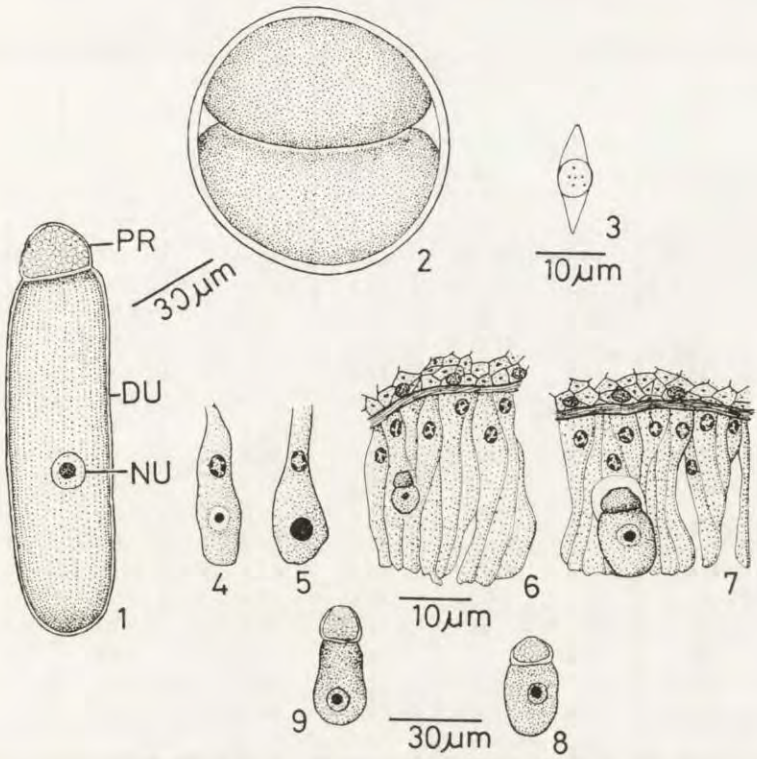

Fig. 1. *1–9* — *Stenoductus polydesmi* sp. n
1 — Sporont, *2* — Gametocyst, *3* — Spore, *4–5* — Intracellular aseptate trophozoites, *6–7* — Intercellular septate trophozoites, *8–9* Lumen trophozoites.
Abbreviations: DU — deutomerite, NU — nucleus, PR — protomerite.

TL = 105.6 to 148.5 (124.1), DW = 26.4 to 66 (45.4);
PL = 13.2 to 19.8 (17.8), PW = 19.8 to 26.4 (21.6);
PL : TL = 1 : 7, PW : DW = 1 : 2.1.

The details of measurements of these specimens are shown in Table 1.

Gametocysts (Fig. 1 *2*): Gametocysts white, opaque, spherical; line of association clear; cyst wall single, of hyaline material, 3.3 μm thick.

Measurements. 77 to 100.8 μm (83.8 μm).

Sporoduct. Sporoduct milky-white, uniformly cylindrical with a central canal. A gametocyst measuring 77 μm, formed a 180 by 27 μm sporoduct.

Spores (Fig. 1 *3*). Spores ovoid, with hyaline epispore forming conical extensions at poles; 4 to 8 dark bodies inside spores; sporozoites vermiform, 8 per spore. Fresh spores measured 14 by 4.2 μm.

Table 1

Measurements (in micrometers) of 20 sporonts of *Stenoductus polydesmi* sp. n.

No.	TL	PL	DL	PW	DW	PL : TL	PW : DW
(1)	105.6	13.2	92.4	19.8	26.4	1 : 8.00	1 : 1.33
(2)	125.4	19.8	105.6	23.1	46.2	1 : 6.33	1 : 2.00
(3)	138.6	19.8	118.8	23.1	49.5	1 : 7.00	1 : 2.14
(4)	141.9	19.8	122.1	23.1	29.6	1 : 7.17	1 : 1.28
(5)	148.5	16.5	132.0	23.1	49.5	1 : 9.00	1 : 2.14
(6)	145.2	16.5	128.7	23.1	59.4	1 : 8.80	1 : 2.57
(7)	128.7	19.8	108.9	26.4	56.1	1 : 6.50	1 : 2.13
(8)	112.2	16.5	95.7	19.8	39.6	1 : 6.80	1 : 2.00
(9)	118.8	16.5	102.3	19.8	33.0	1 : 7.20	1 : 1.67
(10)	128.7	16.5	112.2	19.8	46.2	1 : 7.80	1 : 2.33
(11)	115.5	19.8	95.7	19.8	56.1	1 : 5.83	1 : 2.83
(12)	105.6	16.5	89.1	19.8	39.6	1 : 6.40	1 : 2.00
(13)	122.1	19.8	102.3	23.1	66.0	1 : 6.17	1 : 2.86
(14)	112.2	19.8	92.4	19.8	49.5	1 : 5.67	1 : 2.50
(15)	115.5	19.8	95.7	23.1	42.9	1 : 5.83	1 : 1.86
(16)	108.9	16.5	92.4	19.8	39.6	1 : 6.60	1 : 2.00
(17)	112.2	16.5	95.7	19.8	39.6	1 : 6.80	1 : 2.00
(18)	108.9	16.5	92.4	19.8	39.6	1 : 6.60	1 : 2.00
(19)	145.2	16.5	128.7	23.1	59.4	1 : 8.80	1 : 2.57
(20)	141.9	19.8	122.1	23.1	39.6	1 : 7.17	1 : 1.71

Abbreviation: DL — Deutomerite length, DW — Deutomerite width, PL — Protomerite length, PW — Protomerite width, TL — Total length.

Biology

Gametocysts maintained in moist chamber at room temperature developed and extruded spores in 6 days. The spores came out one after the other through the tip of a well-developed sporoduct.

Spores exposed to the midgut fluid of the host released their sporozoites through one of their poles in 2 min time. Infection appears contaminative, and sporozoites are released in the midgut, obviously under the influence of the midgut fluid. Penetration of the midgut epithelial cells by the sporozoites was not observed.

The smallest observed intracellular trophozoite was spherical (Fig. 1 4) and measured 1.4 μm. The 5 μm long trophozoite was septate, the septum dividing the oval body into a 2.2 μm long protomerite and a 2.8 μm long deutomerite. Deutomerite lodged a spherical nucleus. Trophozoites measuring 14 μm were intercellular or were in the midgut lumen; their protomerites measured 4.2 μm long, deutomerites 9.8 μm. Nuclei with a deep-staining endosome and an unstained extraendosome. Epimerites were not observed in trophozoites.

Host-specificity

The spores of *Stenoductus carlogoni*, *S. trigoniuli*, *S. chondromorphi* and *S. polydesmi* hatched specifically in the midgut fluids of their hosts. The spores of *S. chondromorphi* besides hatched non-specifically in the midgut fluid of *Polydesmus* sp., and of *S. polydesmi* in that of *C. kelaarti*. The sporozoites of these gregarines became active and came out of the spores in 2 to 5 min. Midgut fluid of *Phyllogonostreptus negotiosus* was not able to liberate the sporozoites from the spores of the four gregarine species (Table 2).

Table 2

Midgut fluid	Spores of gregarines			
	Stenoductus carlogoni	*S. trigoniuli*	*S. chondromorphi*	*S. polydesmi*
Carlogonus palmatus	+	—	—	—
Trigoniulus goesi	—	+	—	—
Chondromorpha kelaarti	—	—	+	+
Polydesmus sp.	—	—	+	+
Phyllogonostreptus negotiosus	—	—	—	—

+, indicates dehiscence; —, indicates nondehiscence.

Taxonomic Summary

Diagnosis: Sporonts cylindrical; total length 105 to 148.5 μm (124.1 μm); protomerite hemispherical, broader than long, apical papilla and apical pore absent. Deutomerite elongated, cylindrical; nucleus spherical or slightly ovoid; endosome round; gametocyst spherical, of diameter 77 μm to 100.8 μm (83.8 μm); line of association clear; dehiscence through single sporoduct. Spores ovoid; 14 by 4.2 μm; with hyaline epispore drawn out into conical extensions at poles.

Ratios: PL : TL = 1 : 7; PW : DW = 1 : 2.1.
Host: *Polydesmus* sp.
Location in host: Intestine.
Type locality: Campus of the Sree Narayana College, Cannanore, Kerala, India.
Date of collection: June to September of 1976 and 1977.
Holotype: To be deposited in the Museum of the Zoological Survey of India, 34, Chittaranjan Avenue, Calcutta.

Discussion

The present gregarine from *Polydesmus* sp. morphologically resembles *Stenophora triangula* Hukui. But it differs in its measurements and in having a hemispherical protomerite without apical papilla and apical pore. Gametocyst dehiscence unknown. Spores are also unknown. The gregarines known from *Polydesmus* species are, *Stenophora fontaria* (Crawley) Watson from *Polydesmus* sp., *Amphoroides polydesmi* (Leger) Labbe from *P. complanatus* and *P. dispar*, *A. cerci* Triffitt from *P. complanatus*, *A. ventosa* Tuzet and Guerin from *P. complanatus*, *Hyalosporina rayi* Chakravarty from *Polydesmus* sp. and *Stenophora kampinosi* Lipa and Stachurska from *P. complanatus*. These gregarines show marked difference from the present gregarine. The dehiscence of gametocysts through a single well-formed tubular sporoduct justifies its assignment to the family *Monoductidae*. The known species of *Monoductus* have prong-bearing epimerites, nuclei tethered by myonemes and spores which are truncated, compressed, spindle-shaped and with longitudinal dorsal ridge. The present gregarine have sporonts without epimerites, nuclei without myoneme attachment and ovoid spores with hyaline epispore drawn out into conical extensions at poles. These differences in characters do not justify the assignment of the present species under the genus *Monoductus*. This gregarine resembles the genus *Stenoductus* in having sporonts without epimerites, nuclei without myoneme attachment and spores with hyaline epispore drawn out at poles.

The relatively small size, cylindrical body, hemispherical protomerite without apical papilla and apical pore, and the ovoid spores with hyaline epispore drawn out into conical extensions at poles clearly separate this species from the known species of *Stenoductus*. It is, therefore, considered as a new taxon and is named *Stenoductus polydesmi* sp. n. after its millipede host.

Host-specificity

We made an extensive survey of the cephaline gregarines of the millipedes in Kerala and reported on 10 species of the genera, *Stenophora* and *Stenoductus* (Ramachandran 1976; Janardanan 1978; Janardanan and Ramachandran 1979, 1981 a, b, 1982 a, b). The millipedes, we examined, harboured gregarines. We attribute it to the millipedes' life in soil or leaf-litter where the cysts of gregarines can easily survive, and the spores could be easily swallowed by healthy individuals, as suggested by Lipa (1967). Foerster in 1938, as cited by Lipa (1967), noted that out of the 245 known spec-

ies of gregarines 158 infected single hosts, 44 had two hosts each, and 43 infected 3 or more hosts. Stammer reported in 1957 that among the eugregarines of *Oligochaeta* and Arthropoda 20% infected 2 or more hosts and 7% infected hosts of various families or orders. Host-specificity among gregarines thus ranges from narrow to wide. Our studies on 10 species reveal different levels of host-specificity. Six species are species specific, four infect more than one host and one millipede is infected by 2 species of gregarines (Table 3).

Table 3

Host range of gregarines recorded from millipedes in Kerala

Hosts	Gregarine species									
	Stenophora negotiosus	*Stenoductus carlogoni*	*S. chondromorphi*	*S. xenoboli*	*S. trigoniuli*	*S. organognathi*	*S. polydesmi*	*S. ktenostrepti*	*S. nitida*	*S. disticta*
Sphaerotheridae										
Arthrosphaera dalyi Pocock										+
A. disticta Pocock										+
A. nitida Pocock									+	
Arthrosphaera sp.										+
Spiroboloidea										
Aulacobolus graveleyi Silvestri				+						
Trigoniulus goesi (Porath)					+					
Xenobolus acuticonus Attems				+	+					
Harpagophoridae										
Carlogonus palmatus Demange		+								
Organognathus janardhanani Demange						+				
Ktenostreptus calcaratus Demange								+		
Fageostreptus hyatti Demange						+				
Phyllogonostreptus negotiosus (Carl)	+									
Polydesmoidea										
Chondromorpha kelaarti (Humbert)			+							
Polydesmus sp.							+			

Janardanan and Ramachandran (1979) reported that the hatching of gregarine-spores is a highly specific process, the possible critical host-factor being the host's digestive enzyme(s). The exact role of the enzyme(s) has not been determined. Spores exposed to the host's midgut fluid invariably hatched in 2 to 5 min. The enzyme(s) appears to

trigger off the inherent hatching mechanism of sporozoites. The spores of *Stenoductus carlogoni*, *S. trigoniuli*, *S. chondromorphi* or *S. polydesmi* hatch exclusively in the midgut fluids of their specific hosts. The spores of *S. chondromorphi* besides hatch-specifically in the midgut fluid of *Polydesmus* sp., and of *S. polydesmi* in that of *C. kelaarti*. These non-specifically released sporozoites were unable to establish themselves and develop further. Our experiments thus suggest that host-specificity of these gregarines depends on more factors than on the hatching-enzyme(s), probably on specific physiological and biochemical factors obtainable in the midguts of their specific millipede-hosts.

ACKNOWLEDGEMENTS

The authors express their sincere thanks to Dr. J. M. Demange of the Natural History Museum, Paris, for the taxonomic identification of the millipedes. One of the authors (K.P.J.) is thankful to the U.G.C., New Delhi, for financial assistance.

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BOOKS RECEIVED

A New Series, Synopses of the British Fauna, No. 22. *Edited by* Doris M. Kermack and R. S. K. Barnes

BRITISH AND OTHER FRESHWATER CILIATED *PROTOZOA*

Part I. *Ciliophora* : *Kinetofragminophora*

Key and notes for the identification of the free-living genera

Colin R. CURDS

Published for the Linnean Society of London and The Estuarine and Brackish-water Sciences Association, Cambridge University Press, Cambridge, London, New York, New Rochelle, Melbourne, Sydney, 1982, 387 pp.

CONTENTS: Foreword, Introduction, General structure and biology, Classification of ciliated protozoa, Practical methods, Keys to certain groups of ciliated protozoa, Systematic part: Keys and descriptions of genera of *Gymnostomata*, Keys and descriptions of genera of *Vestibulifera*, Keys and descriptions of genera of *Hypostomata*, Keys and descriptions of genera *Suctoria*, Glossary of special terms, Acknowledgements, References, Taxonomic index

A. V. IVANOV, J. I. POLJANSKY, A. A. STRELKOV

GREAT "PRACTICUM" ON THE INVERTEBRATE ZOOLOGY *Protozoa, Spongia, Coelenterata, Ctenophora, Plathelminthes, Nemertini, Nemethelminthes*

Third edition, revised and enlarged

Moskva, Vyssaja Skola, 1981, p. 504

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Titles of references are given in their original language (not translated). In papers written in English, French or German, the Cyrillic type of the Russian references is translitterated according to the international system (ISO Recomendation R 9 September). This regulation is not applied to name if there exists in their traditional spelling. Also author may freely choose in transliteration of his name. In Russian papers, the Russian references are cited in Cyrillic, the others in the Latin characters, but they must be listed all together in the Latin alphabetical order.

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In preparation:

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