Inhibition of Flagellum Morphogenesis in the True Slime Mould *Didymium nigripes*

By SYLVIA J. KERR*

*Department of Zoology, University of Minnesota, Minneapolis, 55455 and
Department of Biology, Augsburg College, Minneapolis, 55404, U.S.A.*

(Received 9 November 1971; revised 24 April 1972)

SUMMARY

Flagellum morphogenesis in the slime mould, *Didymium nigripes*, involves a sequence of events during which amoebae are changed into biflagellated cells; in a population of cells this transformation is nearly synchronous. In the present study a series of inhibitors thought to inhibit RNA and protein synthesis and microtubule assembly were added in an attempt to characterize the macromolecular events associated with this amoeboid-flagellate transformation.

High concentrations of acriflavin and proflavin (50 µg/ml) and lower concentrations of cycloheximide (5 to 10 µg/ml) blocked the morphogenetic process completely. Other reported inhibitors of RNA synthesis and microtubular assembly delayed the onset of flagellum formation for varying periods of time dependent upon the concentrations employed.

INTRODUCTION

Myxamoebae of the true slime mould, *Didymium nigripes*, which are just entering the stationary phase of growth, readily differentiate into biflagellated cells if they are washed free of bacteria and suspended in 0.05 M-phosphate buffer, pH 6.5. This morphogenetic process is quite synchronous and includes several stages (Kerr, 1960). Development and elongation of the flagella is described by Kerr (1972). Previous studies (Kerr, 1965) have established that morphogenesis is blocked by addition of streptomycin (100 µg/ml), 2,4 dinitrophenol (10^{-4} M), cycloheximide (10 µg/ml) and acriflavin (50 µg/ml).

The current investigation focuses on the question of whether macromolecular events, such as RNA and protein synthesis, are prerequisites for flagellum morphogenesis and on an attempt to sort out the approximate period of time during the incubation period when inhibition of these processes results in blockage of cellular differentiation.

METHODS

Preparation of amoebae. C-6 amoebae were grown from spores on glucose-peptone-yeast extract agar with brucine (Kerr, 1960) in the presence of *Aerobacter aerogenes* for 4 days at 21 °C. When the plates were beginning to clear of bacteria the amoebae were harvested in deionized water and washed free of bacteria by sedimenting them five times in a centrifuge at 2000 g.

Incubation conditions. Suspensions of amoebae (5 to 10 ml in a 50 ml beaker) were incubated at 21 °C at a concentration of 1×10^6 amoebae/ml in 0.05 M-phosphate buffer (0.83 g K_2HPO_4 + 1.36 g KH_2PO_4 /l deionized water), pH 6.5. Periodically the samples

* Present address: 1571 Burton, St Paul, Minnesota, 55108, U.S.A.

were mixed and a drop of the suspension was withdrawn and observed under the phase-contrast microscope. The number of flagellated cells/50 cells was recorded.

Inhibitors tested. The following compounds were tested for their ability to inhibit flagellum morphogenesis: Actinomycin D (Sigma Chemical Company, St Louis, Missouri, U.S.A.); 8-azaguanine (General Biochemical, Chagrin Falls, Ohio, U.S.A.); 5-fluorouracil (Hoffman-Roche, Nutley, New Jersey, U.S.A.); acriflavin (Mann, New York, New York, U.S.A.); proflavin (Allied Chemical from Fisher Scientific, Chicago, Illinois, U.S.A.); colchicine (Sigma); velban (Grand Island Biological, Grand Island, New York, U.S.A.); isopropyl-*n*-phenyl carbamate (Sigma); ethidium bromide (Sigma); streptomycin sulphate (Squibb, New York, New York, U.S.A.); and cycloheximide (Sigma). Concentrations used are specified in Table 1.

RESULTS

Synchrony of the population

The synchrony of the morphogenetic process in populations of amoebae was evaluated in the first 100 populations studied. In these populations none of the amoebae were flagellated after the washing procedure, at the onset of incubation. Mean values obtained for flagellation in each 50 amoebae counted at the stated intervals from onset of the incubation period were: 30 min – 8] flagellated (s.d. = 6.7); 45 min – 27 flagellated (s.d. = 10); and 60 min – 47 flagellated (s.d. = 2.25). Data from populations of amoebae was not included in the analysis if the number of flagellated amoebae observed at any of these times differed by more than 1 s.d. from the mean, except for the 60 min value where populations with all 50 amoebae flagellated were retained.

Pre-incubation temperature

An attempt was made to determine whether chilling of the amoebae during the washing procedure might play a role in the time course of flagellum formation. Routinely amoebae were washed and centrifuged at 4 °C before incubation at 21 °C; the time of exposure to cold ranged from 12 to 15 min. Other populations of amoebae were washed and centrifuged at 21 °C before incubation. No difference in the time-course of flagellation was noted in amoebae treated in these two ways. Extending the time of cold exposure to 2 h before the 21 °C incubation similarly did not effect the course of flagellation.

Inhibitor studies – RNA synthesis

Acriflavin, proflavin, actinomycin D, 8-azaguanine and 5-fluorouracil were used to test for a requirement for RNA synthesis during flagellum formation. The effect of various concentrations of these compounds added at the beginning of incubation to populations of amoebae is summarized in Table 1. During the period of observation (2 to 5 h), complete inhibition of flagellation was obtained only in the presence of acriflavin or proflavin (50 µg/ml). High concentrations of actinomycin D, 8-azaguanine and 5-fluorouracil were effective in delaying the appearance of flagella (see Table 1). The effect of these latter compounds was evaluated in terms of delay of flagellum formation in the population of amoebae, i.e. if all of the amoebae in a particular inhibitor became flagellated after 90 min of incubation, this represented a 30 min delay in comparison to the untreated population which all became flagellated after 60 min. Non-inhibitory concentrations of EDTA were added at the same time as these compounds in an attempt to increase their uptake, but no enhancement of the inhibitory effect of these compounds was noted.

Table 1. *Effect of various compounds on flagellum morphogenesis*

Inhibitor	Concentration	Time when 90 % of amoebae were flagellated (normally at 60 min)
Auxiliary compounds		
EDTA in phosphate buffer	10^{-4} to 10^{-7} M 5×10^{-4} M 10^{-3} M	No delay 20 to 30 min delay Amoebae encysted; no flagellation
5 % Ethanol in phosphate buffer		No delay
0.1 % DMSO in phosphate buffer		30 min delay
Compounds thought to affect RNA synthesis		
Actinomycin D (dissolved in ethanol) in phosphate buffer	50 μ g/ml 100 μ g/ml 200 μ g/ml	No delay 15 min delay 30 min delay
Actinomycin D (as above) in 0.1 % DMSO	100 μ g/ml 200 μ g/ml	45 min delay 45 to 60 min delay
Actinomycin D (as above) in 10^{-7} to 10^{-5} M-EDTA	50 μ g/ml 100 μ g/ml 200 μ g/ml	No delay 20 to 30 min delay 30 min delay
8-Azaguanine in phosphate buffer or in buffer with 10^{-6} to 10^{-4} M-EDTA	0.01 mg/ml 0.1 mg/ml 0.5 mg/ml 1.0 mg/ml	No delay 15 min delay 30 to 40 min delay Amoebae lysed
5-Fluorouracil in phosphate buffer	2×10^{-3} to 2×10^{-4} M 2×10^{-5} to 2×10^{-6} M 2×10^{-3} M	15 min delay 20 to 30 min delay No delay for population but first flagella appeared later
5-Fluorouracil (as above) in 10^{-4} M-EDTA	2×10^{-4} to 2×10^{-5} M 2×10^{-6} M 2×10^{-5} to 2×10^{-6} M	15 min delay; first flagella appeared later No delay; first flagella appeared later 0 to 15 min delay; first flagella appeared later
Acriflavin in phosphate buffer	10 to 25 μ g/ml 50 μ g/ml	No delay Inhibition
Proflavin in phosphate buffer	10 μ g/ml 25 μ g/ml 50 μ g/ml	15 to 20 min delay 40 to 45 min delay Inhibition
Compounds thought to affect protein synthesis		
Cycloheximide	0.5 to 2.5 μ g/ml 5 μ g/ml 7.5 μ g/ml 10 to 15 μ g/ml	No inhibition Inhibition in 80 to 100 % of population Inhibition in 100 % of population Inhibition and toxic effects (encystation)
Compounds thought to affect microtubular assembly		
Colchicine in phosphate buffer or in buffer with 10^{-5} to 10^{-4} M-EDTA	0.01 to 1.0 mg/ml 2 mg/ml 5 mg/ml 10 mg/ml	No delay 30 min delay 45 min delay; 30 min delay before first flagella appeared 40 to 75 min delay
Velban (vinblastine sulphate) in phosphate buffer	0.001 to 0.005 μ g/ml 0.01 to 0.1 μ g/ml 0.5 μ g/ml	No delay 15 min delay 45 min delay; 30 min delay before first flagella appear
Iso-propyl- <i>n</i> -phenyl carbamate (dissolved in ethanol) in phosphate buffer	2.5 μ g/ml 25 μ g/ml	No delay 30 min delay
Other compounds		
Ethidium bromide in phosphate buffer	1 to 10 μ g/ml 25 to 50 μ g/ml 100 μ g/ml	No delay 15 min delay 45 min delay
Streptomycin sulphate in phosphate buffer	250 μ g/ml 500 μ g/ml	45 min delay 75 min delay

Fig. 1. Effect of addition of cycloheximide to amoebae undergoing morphogenesis. Cycloheximide addition delayed for varying periods of time (horizontal axis) resulted in differing numbers of cells undergoing flagellation (vertical axis).

Definition of the period of time when the amoebae were sensitive to inhibition of RNA synthesis was attempted by postponing the addition of effective concentrations of acriflavin, proflavin and 8-azaguanine until 10, 15, 20, 25 or 30 min after the commencement of normal incubation. Acriflavin and proflavin were completely inhibitory when added during the first 30 min. When addition of 8-azaguanine was postponed for 10, 20 or 30 min, the delay before the amoebae became flagellated was decreased as addition was delayed.

Protein synthesis

Cycloheximide, a reported inhibitor of protein synthesis, completely and reversibly inhibited flagellation without obvious damage to the amoebae. Inhibition of flagellation continued as long as the amoeba remained in a solution of cycloheximide. The period of maximum sensitivity to cycloheximide was determined by splitting a population of amoebae into eight portions, postponing addition of cycloheximide (5 µg/ml) until after 5, 10, 15, 20, 25, 30 or 60 min of incubation, and determining the number of amoebae per 50 cells which were flagellated at 60 min. Cycloheximide added during the first 10 min of incubation exhibited a maximum inhibitory effect and sensitivity to the compound decreased as incubation time before addition was increased (Fig. 1).

When amoebae inhibited from forming flagella by cycloheximide were sedimented by spinning at 5000 g and then resuspended in phosphate buffer at 21 °C without cycloheximide, they all grew flagella. Recovery from cycloheximide inhibition was studied in a series of experiments where cycloheximide (7.5 µg/ml) was added to a population of amoebae at the beginning of the incubation period, washed out of separate portions of these populations at intervals, and the number of flagellated amoebae per 50 amoebae was counted at 15 min intervals. Amoebae exposed to cycloheximide in this manner and resuspended in phosphate buffer always showed delayed flagellation in comparison to that

Fig. 2. Delay in appearance of flagellated amoebae after treatment with $7.5 \mu\text{g}$ cycloheximide/ml for varying periods of time. Control – amoebae incubated under standard conditions; CW, control amoebae subjected to cold washing procedure; WO-0, WO-10, WO-15, WO-20, WO-30, WO-45 cells placed in $7.5 \mu\text{g}$ cycloheximide/ml at the beginning of incubation and then subsequently removed from cycloheximide, e.g. WO-10: cells placed in cycloheximide at time 0 and removed from cycloheximide after 10 min.

of amoebae not exposed to cycloheximide (Fig. 2). The behaviour of amoebae after exposure to cycloheximide can be summarized as follows: (i) the delay in appearance of flagella caused by the cold wash is almost identical to a 5 min difference in incubation time in cycloheximide later in the experiment (e.g. comparing 15 and 20 min of incubation before washout). Thus the amount of delay caused by the cold treatment seems to be directly proportional to the amount of time spent in the cold; (ii) amoebae exposed to cycloheximide briefly (0 time washout – in reality cycloheximide exposure up to 1 min could have occurred before pelleting) were delayed proportionately longer than their exposure to cycloheximide. The observed delay was proportional to about 10 min of exposure to cycloheximide later in the time course of morphogenesis; (iii) amoebae exposed to cycloheximide for longer intervals showed additional delay in flagellation after washout directly proportional to the length of exposure to cycloheximide.

Microtubular assembly

Colchicine and velban (vinblastine sulphate), inhibitors of microtubular assembly in other organisms, were tested for their ability to block flagellum production. At high concentrations flagellum production was delayed but not blocked (Table 1). Addition of non-inhibitory concentrations of EDTA in the presence of colchicine did not alter the effectiveness of colchicine.

Isopropyl-*n*-phenyl carbamate (IPC), a mitotic inhibitor thought to dissociate microtubules, was tested in a series of delayed addition and wash out experiments. IPC ($25 \mu\text{g}/\text{ml}$) added during the first 20 min of incubation delayed flagellation in the population for 30 min. If this concentration was added during the next 15 to 20 min before flagellates appeared in the untreated population, morphogenesis of half the cells in the population

was delayed for 30 min. If added after the population was flagellated, the flagella remained intact.

When IPC was added at the beginning of incubation and removed by washing the amoebae at 5000 *g* and then resuspending them in phosphate buffer, the inhibitory effect was reversed, but no delay in flagellum morphogenesis occurred when IPC was removed immediately. When IPC remained in the incubation medium for 15 min or longer before removal, the percentage of amoebae showing inhibition after 60 min of incubation progressively increased. Populations of cells exposed to IPC for varying periods of time were uniformly flagellated after 90 min of incubation.

Other inhibitors

Ethidium bromide, an inhibitor of mitochondrial nucleic acid synthesis in other systems, when tested at concentrations of 25 and 50 $\mu\text{g/ml}$, produced a slight delay in morphogenesis, and at 100 $\mu\text{g/ml}$ flagellation was delayed for about 45 min. When addition of 100 $\mu\text{g/ml}$ was postponed, delay in flagellation was decreased, e.g. at 10 min the delay lasted 30 min and at 20 min the delay lasted only 15 min. At 30 min there was no effect on flagellation.

Amoebae incubated in the presence of streptomycin (250 to 500 $\mu\text{g/ml}$) did not become flagellated during the first hour of incubation but did eventually become flagellated. As streptomycin concentration was increased, the delay before the appearance of flagella was also increased (Table 1).

In one series of experiments streptomycin (250 $\mu\text{g/ml}$) was added once at the beginning of incubation and again at various intervals in the morphogenetic process. The number of flagellated amoebae per 50 amoebae was scored at intervals and compared with amoebae which received single doses of streptomycin (either 250 $\mu\text{g/ml}$ or 500 $\mu\text{g/ml}$) at the beginning of incubation. If 250 $\mu\text{g/ml}$ were added at time 0 and again after 15, 45 or 60 min, the amoebae behaved like amoebae which had received a single dose of 250 $\mu\text{g/ml}$ at the beginning of incubation. If the second dose of 250 $\mu\text{g/ml}$ was added 30 min after the beginning of incubation, the degree of flagellation was the same as if 500 $\mu\text{g/ml}$ had been added at the beginning of incubation. This special sensitivity of cells to additional streptomycin at 30 min of incubation suggests that recovery from streptomycin inhibition may be correlated with destruction or neutralization of the compound by the amoebae.

DISCUSSION

Synchronous differentiation of cells in a eukaryotic system which can be easily manipulated provides a relatively rare and valuable opportunity to study macromolecular events in cellular differentiation. Flagellum synthesis and assembly have been studied previously in several normally flagellated organisms: *Ochromonas danica*, *Astasia longa*, *Euglena gracilis* (Rosenbaum & Child, 1967); *Peranema trichophorum* (Tamm, 1967); and *Chlamydomonas reinhardtii* (Rosenbaum, Moulder & Ringo, 1969). In several of these systems flagellum synthesis was blocked in the presence of cycloheximide and colchicine; Rosenbaum *et al.* have conclusively shown that flagellum synthesis in *Chlamydomonas* can be separated into stages of protein synthesis (blocked by cycloheximide) and microtubular assembly (blocked by colchicine).

The amoeboid-flagellate transformation which occurs in the true slime moulds and in some soil amoebae, e.g. *Naegleria gruberi* (see Fulton & Dingle, 1967) involves not only organellar growth but also differentiation of amoebae into a new morphological type.

Flagellum morphogenesis in *N. gruberi* is blocked early during incubation by the presence of actinomycin D (Wade & Satir, 1968), suggesting that induction of morphogenesis in this system requires RNA synthesis. Failure of routinely employed concentrations of actinomycin D to block flagellum morphogenesis in *Didymium nigripes* cannot be construed as evidence that DNA-dependent RNA synthesis is not involved in the morphogenetic process because acriflavin and proflavin do block morphogenesis. Considering that the natural habitat of these organisms is in soil where antibiotic-producing organisms are often found, it is not unreasonable to postulate that they have evolved some means of destroying the effectiveness of this compound or of excluding it from the cells. This view is supported by the observation that *D. nigripes* can grow and divide in the presence of actinomycin D (Kerr, 1965) and that the plasmodial stage of *Physarum polycephalum*, a closely related slime mould, has also been shown to be relatively insensitive to actinomycin D (see Rusch, 1970). Proflavin, one of the inhibitors effective in blocking flagellation in *D. nigripes*, has been shown to inhibit [H^3]uridine uptake into RNA fractions of plasmodia of *Physarum polycephalum* specifically at concentrations of 25 $\mu\text{g/ml}$ (Sauer, Babcock & Rusch, 1969).

Cycloheximide inhibition of flagellum morphogenesis strongly suggests that protein synthesis is involved in the amoeboid-flagellate transformation. Typically the greatest effect of low concentrations of cycloheximide is to block the incorporation of amino acids into protein (Siegel & Sisler, 1963; Ennis & Lubin, 1964; Cummins, Brewer & Rusch, 1965; Rosenbaum *et al.* 1969). Attempts to link cycloheximide to inhibition of respiratory metabolism in *Euglena gracilis* have been unsuccessful (Kirk, 1970). Cycloheximide added to differentiating amoebae of *Didymium nigripes* immediately halted flagellar elongation; thus it seems probable that its effect in this system is to block synthesis of protein required either for flagellum synthesis or assembly.

Another process in flagellum production which has been studied in other systems is the assembly of precursors into microtubular arrays. Microtubule assembly in *Chlamydomonas reinhardtii* is reported to be blocked by high concentrations of colchicine (Rosenbaum *et al.* 1969). This is thought to be due to the binding of colchicine to microtubular proteins (Borisy & Taylor, 1967). However in *Naegleria gruberi* concentrations of colchicine up to 5×10^{-2} M failed to block or delay flagellum formation (Yuyama, 1971), but mercaptoethanol did block flagellum morphogenesis (Wade & Satir, 1968). In ciliary regenerating systems of *Stentor coeruleus* concentrations from 4 to 2×10^{-4} M-isopropyl-*n*-phenyl carbamate were capable of reversibly blocking membranellar band regeneration (Banerjee & Margulis, 1969). Delay of morphogenesis, not total inhibition of microtubule assembly, was found when similar compounds were tested on *Didymium nigripes*.

Mitochondrial function may play an important role in flagellum morphogenesis. Schuster (1965) described changes in the mitochondrial structure of *Didymium nigripes* associated with the amoeboid-flagellate transformation. Kerr (1965) reported inhibition of flagellation in the same organism if mitochondrial oxidative phosphorylation was blocked by the presence of 10^{-4} M-2,4 dinitrophenol or by incubating the cells in a N_2 atmosphere. Delay of flagellation induced by ethidium bromide during the early stages of incubation may also suggest mitochondrial involvement, since 10 $\mu\text{g/ml}$ specifically blocks DNA replication in plasmodial mitochondria of the slime mould, *Physarum polycephalum* (Horwitz & Holt, 1971). Perhaps mitochondrial replication or transformation is one of the early steps required for flagellum differentiation.

Thus it seems that several processes involved in flagellum morphogenesis can be identified through the use of compounds whose mode of inhibition has been defined in other systems.

Confirmation of the mode of action in *Didymium nigripes* is impractical until this organism can be grown satisfactorily in axenic culture. When comparing time of release of inhibition (determined by delayed-addition experiments) a tentative time sequence for events involved in flagellum morphogenesis can be constructed. Steps inhibited by streptomycin and ethidium bromide are important during the first 10 min of incubation and become less important thereafter. Involvement of ethidium bromide in mitochondrial-DNA synthesis and of streptomycin in 70 S ribosomal-protein synthesis suggest that mitochondrial change may be important at this stage. The importance of cellular-RNA synthesis, as demonstrated by proflavin inhibition, is prominent during the first 20 min of incubation and declines in importance thereafter. Sensitivity of the morphogenetic process to cycloheximide, presumably an inhibitor of protein synthesis, begins to decline after the first 15 min of incubation. However, cycloheximide added after flagella have appeared will immediately halt elongation (Kerr, 1972). Total sensitivity to isopropyl-*n*-phenyl carbamate does not begin to decrease until after 20 min of incubation has elapsed; during this period of time flagellar protein has been synthesized to allow production of visible flagella in some cells (Fig. 1). Thus presumably the IPC-sensitive step follows that stage of protein synthesis.

I wish to thank Dr Norman Kerr, Department of Zoology, University of Minnesota, and Drs Ralph Sulerud and Neal Thorpe, Department of Biology, Augsburg College, for use of their laboratories and Mrs Lyn Steere for assistance in the preparation of the Figures.

REFERENCES

- BANERJEE, S. & MARGULIS, L. (1969). Reversible inhibition of cilia regeneration in *Stentor coeruleus* by isopropyl-*n*-phenyl carbamate. *Nature, London* **224**, 180-181.
- BORISY, G. G. & TAYLOR, E. W. (1967). The mechanism of action of colchicine. Colchicine binding to sea urchin eggs and the mitotic apparatus. *Journal of Cell Biology* **34**, 535-547.
- CUMMINS, J. E., BREWER, E. N. & RUSCH, H. P. (1965). The effect of actidione on mitosis in the slime mold *Physarum polycephalum*. *Journal of Cell Biology* **27**, 337-341.
- ENNIS, H. L. & LUBIN, M. (1964). Cycloheximide: Aspects of inhibition of protein synthesis in mammalian cells. *Science, New York* **146**, 1474-1476.
- FULTON, C. & DINGLE, A. D. (1967). Appearance of the flagellate phenotype in populations of *Naegleria* amoebae. *Developmental Biology* **15**, 165-191.
- HORWITZ, H. B. & HOLT, C. E. (1971). Specific inhibition by ethidium bromide of mitochondrial DNA synthesis in *Physarum polycephalum*. *Journal of Cell Biology* **49**, 546-553.
- KERR, N. S. (1960). Flagella formation by myxamoebae of the true slime mold, *Didymium nigripes*. *Journal of Protozoology* **7**, 103-108.
- KERR, N. S. (1965). Inhibition by streptomycin of flagella formation in a true slime mold. *Journal of Protozoology* **12**, 276-278.
- KERR, S. J. (1972). Flagellum growth and regeneration in the true slime mould *Didymium nigripes*. *Journal of General Microbiology* **72**, 429-437.
- KIRK, J. T. O. (1970). Failure to detect effects of cycloheximide on energy metabolism in *Euglena gracilis*. *Nature, London* **226**, 182.
- ROSENBAUM, J. L. & CHILD, F. M. (1967). Flagellar regeneration in protozoan flagellates. *Journal of Cell Biology* **34**, 345-364.
- ROSENBAUM, J. L., MOULDER, J. E. & RINGO, D. (1969). Flagellar elongation and shortening in *Chlamydomonas*. *Journal of Cell Biology* **41**, 600-619.
- RUSCH, H. P. (1970). Some biochemical events in the life cycle of *Physarum polycephalum*. In *Advances in Cell Biology*, vol. 1, pp. 297-328. Edited by D. M. Prescott, L. Goldstein & E. McConkey, New York: Appleton-Century-Crofts.
- SAUER, H. W., BABCOCK, K. L. & RUSCH, H. P. (1969). Sporulation in *Physarum polycephalum*: A model system for studies on differentiation. *Experimental Cell Research* **57**, 319-327.

- SCHUSTER, F. L. (1965). Ultrastructure and morphogenesis of solitary stages of true slime molds. *Protistologica* **2**, 49–62.
- SIEGEL, M. R. & SISLER, H. D. (1963). Inhibition of protein synthesis *in vitro* by cycloheximide. *Nature, London* **200**, 675–676.
- TAMM, S. L. (1967). Flagellar development in the protozoon *Peranema trichophorum*. *Journal of Experimental Zoology* **164**, 163–186.
- WADE, J. & SATIR, P. (1968). The effect of mercaptoethanol on flagellar morphogenesis in the amoeboid flagellate *Naegleria gruberi* (Schardinger). *Experimental Cell Research* **56**, 81–92.
- YUYAMA, S. (1971). The effects of selected chemical agents on the amebaflagellate transformation in *Naegleria gruberi*. *Journal of Protozoology* **18**, 337–343.