*J. Cell Sci.* 4, 171–178 (1969)
*Printed in Great Britain*



# THE EFFECT OF ENUCLEATION ON FLAGELLAR REGENERATION IN THE PROTOZOON *PERANEMA TRICHOPHORUM*

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SUMMARY

A rotocompressor was used to enucleate the flagellate protozoon *Peranema trichophorum* at known stages in the mitotic cycle. This new enucleation technique, combined with recently devised methods for amputating the flagellum and recording its regeneration in single living cells, permitted the investigation of the role of the nucleus in flagellar regeneration at different cell ages.

The flagellar regeneration capacity of an enucleate *Peranema* depended on the stage in the cell cycle when the nucleus was removed. Post-division enucleate cells regenerated about half the length reached by sham-operated controls, and at slower rates, while predivision enucleate cells regenerated flagella equally as well as the controls.

Therefore, the nucleus is making an immediate contribution to flagellar regeneration early in the cell cycle, but not late in the cell cycle.

## INTRODUCTION

Enucleation is a classic experimental method for investigating interactions between nucleus and cytoplasm during various cell functions. The effects of enucleation on many aspects of cell morphogenesis have been studied extensively by cutting unicellular organisms into nucleate and enucleate fragments. The general conclusion has emerged from these merotomy experiments that the nucleus (macronucleus in ciliate protozoans) is essential for regeneration and restoration of normal body form (see reviews by Balamuth, 1940; Brachet, 1961; Hammerling, 1953; Weisz, 1954).

The purpose of the present work is to investigate the role of the nucleus in the regeneration of a specific cellular organelle, the flagellum. The previous merotomy experiments could not attack this problem because the enucleation procedure itself served as the amputative operation.

A new technique has been developed for removing the nucleus with very little cytoplasm from the protozoan flagellate *Peranema trichophorum* at known stages in the cell cycle. This new enucleation method, combined with recently devised methods for amputating the flagellum and recording its regeneration in single living cells (Tamm, 1967), now permits investigation of the effects of enucleation on flagellar regeneration at different times in the cell cycle.

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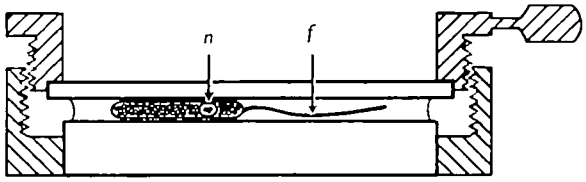


Fig. 1. Diagrammatic cut-away view from the side of the rotocompressor containing a *Peranema* flattened to about the diameter of the nucleus. The cell is in a drop between two circular pieces of glass (not cross-hatched) mounted in separate metal holders (cross-hatched) which screw together. The size of the *Peranema* (and the metal threads) relative to the size of the rotocompressor is greatly exaggerated in this diagram and in Fig. 2. (*f*, flagellum; *n*, nucleus.)

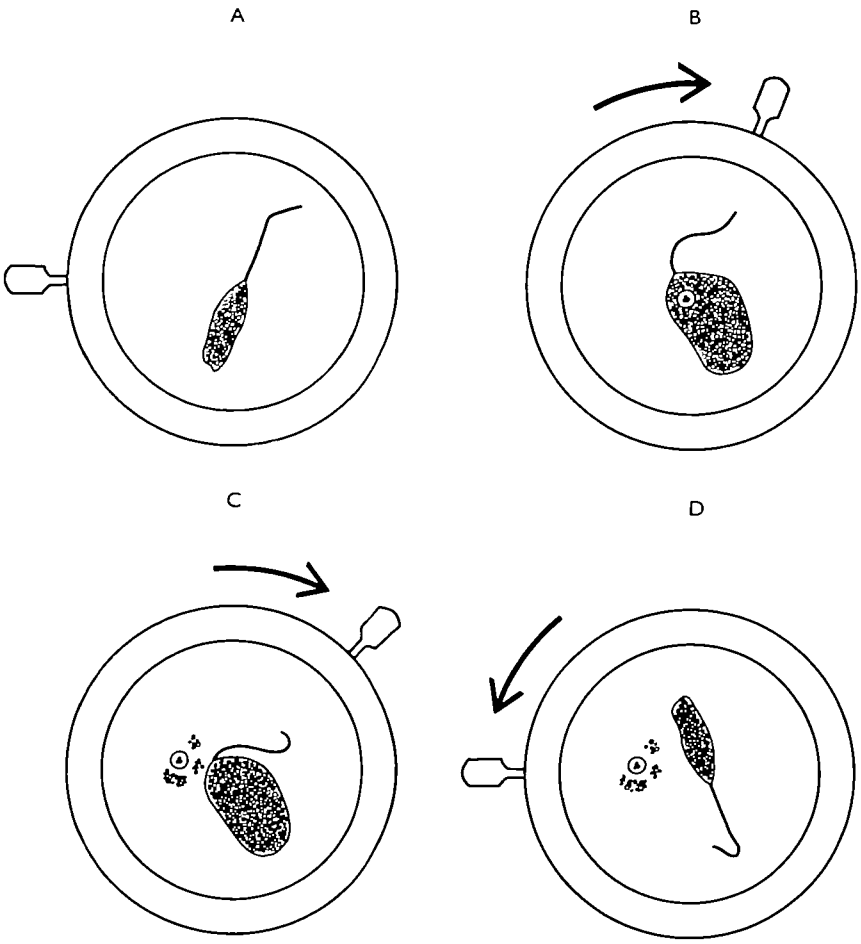


Fig. 2. Enucleation method using the rotocompressor. Diagrammatic view from the top, as observed under the microscope. Rotation of the top part of the rotocompressor is indicated by the arrows and positions of the knob: clockwise rotation compresses the cell, counterclockwise rotation releases pressure. The amount of rotation at different steps is shown only qualitatively. See Materials and Methods for explanation of steps.

## MATERIALS AND METHODS

A full description of the methods used to culture the euglenoid flagellate *Peranema trichophorum*, handle and stage cells, amputate the flagellum, and record and measure flagellar length, has already been published (Tamm, 1967) and will be referred to only briefly here. The new enucleation method is described in detail.

*Peranema* were maintained in axenic stock cultures under controlled conditions of growth. Cells were used at two different stages in the cell cycle, the generation time being about 24 h. Post-division cells of known age were obtained by selecting dividing cells and recording the time of separation of the daughter cells as age zero. The post-division cells were usually 1–4 h old at the time of enucleation. Predivision cells 15–19 h old at enucleation were obtained by selecting large-sized cells. Previous experience in choosing populations of such cells and noting the time of subsequent division showed that this method was reliable for estimating cell age (Tamm, 1967).

The enucleation method is diagrammed in Figs. 1 and 2 (see Figs. 4 and 5 also). A post-division or predivision cell was placed in a small drop in a rotocompressor chamber (Biological Institute, Philadelphia, Penn.) and observed under a phase-contrast microscope at ×250 (Fig. 2A). The cell was gradually compressed to about the diameter of the nucleus, which was then clearly visible as a light grey sphere containing several dark endosomes, or nucleoli (Fig. 2B). Slight further compression almost always resulted in the extrusion of a small amount of protoplasm through a narrow opening at the anterior end of the cell (probably the cytostome) (Figs. 2C, 4). This outflow ceased by itself almost immediately. If the nucleus happened to be at the anterior end, it was often ejected along with a small amount of cytoplasm. Pressure was then released and the extruded contents photographed. The nucleus outside the cell was easily identified (Figs. 2D, 5). The cell was then transferred to a depression slide for further work.

Sham-operated controls were cells in which a comparable amount of cytoplasm alone was removed by this method. Control cells were easily obtained, but only about 20% success in enucleation was achieved.

The leading flagellum of a cell in a depression slide was amputated with a fine tungsten needle operated by a micromanipulator, and flagellar regeneration by the living cell was recorded photographically. Flagellar lengths were measured directly from the photographs.

## RESULTS

The effects of enucleation on flagellar regeneration of typical post-division and predivision cells are shown in Fig. 3. These curves are representative of measurements made on six enucleate and eight control post-division cells, and five enucleate and four control predivision cells. The flagella of all cells were amputated within 30 min after the rotocompressor treatment.

The regeneration kinetics of enucleate and control cells of both age groups consist of an initial lag period during which no elongation can be detected, followed by

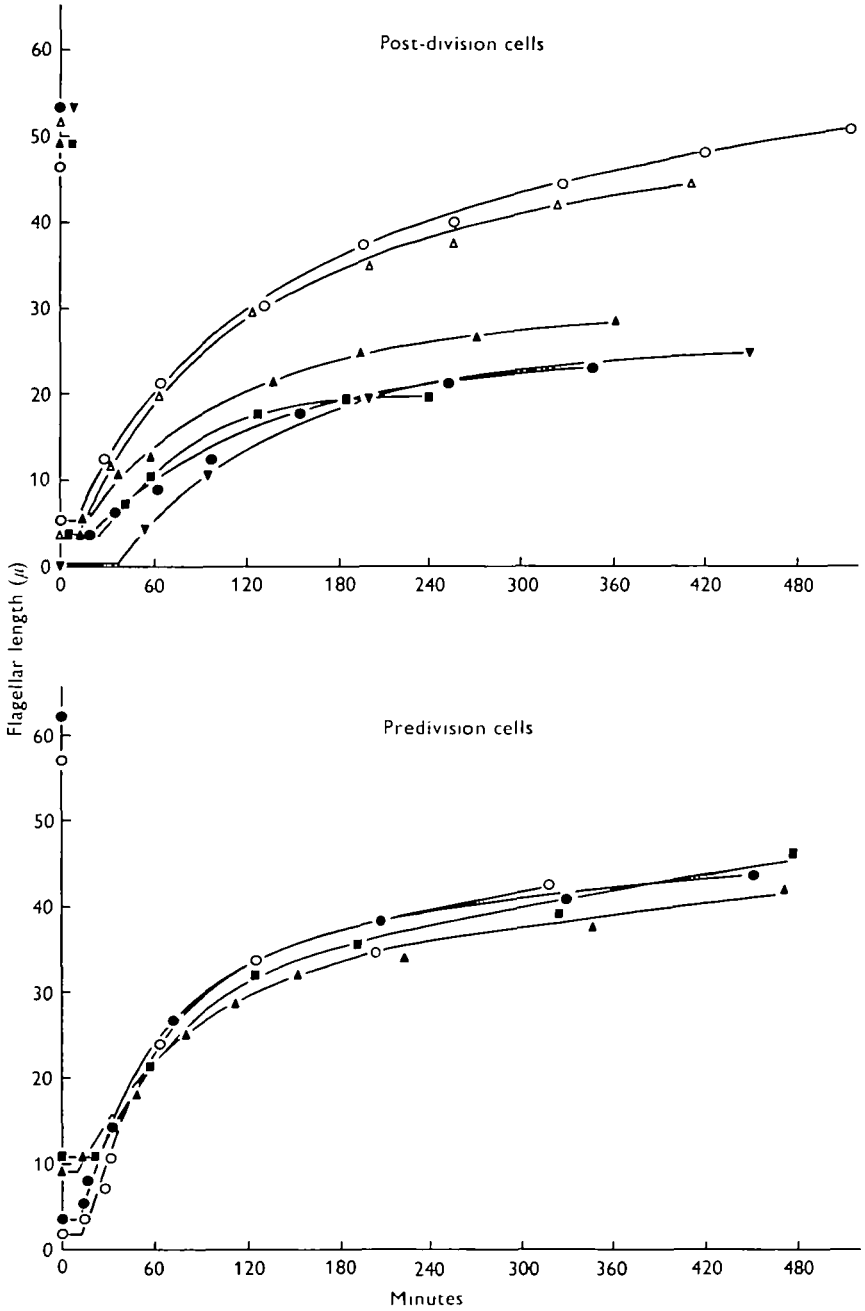


Fig. 3. Effects of enucleation on flagellar regeneration in typical post-division and predivision cells. Open symbols are sham-operated control cells, and closed symbols are enucleate cells. The flagella of all cells were amputated at zero time on the abcissa, within 30 min after the rotocompressor treatment. Flagellar length before amputation is indicated on the ordinate. See Materials and Methods for details of staging cells.

elongation at deceleratory rates. This general pattern also characterizes the flagellar regeneration kinetics of non-compressed cells (normal cells not subjected to the rotocompressor treatment) (Tamm, 1967).

Post-division enucleate cells have a decreased regeneration capacity compared with the control cells (Fig. 3). At any given flagellar length, the elongation rate of the post-division enucleate cells is less than that of the control cells, and by 6–7 h after amputation the flagella of the enucleate cells are only about one-half the length of the control cell flagella. However, the lag period of most of the post-division enucleate cells is not appreciably different from that of the control cells.

In predivision cells there is no detectable difference between the regeneration kinetics of enucleate and control cells (Fig. 3).

Compared with the flagellar regeneration of non-compressed cells (Tamm, 1967), the regeneration kinetics of the rotocompressor-treated cells show more variability within any group. Although the post-division and predivision categories included a 3–4 h range in cell ages (see Materials and Methods), none of this variability could be correlated with the exact age of a cell at the time of the rotocompressor treatment. The regeneration kinetics of post-division control cells, and predivision enucleate and control cells, are fairly similar to the regeneration kinetics of non-compressed cells of the corresponding ages. It was reported previously with non-compressed cells that at similar flagellar lengths the regeneration rate of predivision cells is greater than that of post-division cells (Tamm, 1967). This same difference in regeneration rates between cells of different ages is also apparent in rotocompressor-treated cells (compare post-division control cells with predivision enucleate or control cells in Fig. 3).

In some preliminary experiments, the regeneration capacity of post-division and predivision cells has been tested as a function of the time elapsing between enucleation and flagellar amputation. In post-division cells, flagellar amputation performed at 2 h after enucleation in one cell, and at 3 h after enucleation in another cell, resulted in regeneration kinetics similar to those obtained when flagella were amputated within 30 min following enucleation. In predivision cells there was also no detectable difference in regeneration capacity between cells whose flagella were amputated immediately after enucleation and two cells whose flagella were amputated two hours after enucleation.

No predivision enucleate cell divided or underwent any of the flagellar changes associated with division (shortening of the parental flagellum, growth of two new flagella (Tamm, 1967)). Most of the predivision control cells divided normally.

Post-division and predivision enucleate cells usually lived for several days (in one case more than a week) and exhibited normal locomotory behaviour. Normal locomotion has been observed also in enucleate *Euglena* formed by miscleavage (Leedale, 1959).

## DISCUSSION

This investigation has shown that the flagellar regeneration capacity of an enucleate *Peranema* depends on the stage in the cell cycle when the nucleus is removed: post-

division enucleate cells regenerate about half the length reached by the control cells, and at slower rates; while predivision enucleate cells regenerate flagella equally as well as the control cells.

This means that early in the cell cycle the nucleus is making an immediate contribution to flagellar regeneration. However, in post-division cells the nucleus is not essential for the initiation of elongation, a normal lag period, nor the deceleratory pattern of the kinetics.

In contrast, late in the cell cycle concomitant nuclear activity is not required for any aspect of flagellar regeneration.

Therefore, during the cell cycle flagellar regeneration becomes increasingly independent of the immediate presence of the nucleus. The regeneration kinetics of non-compressed cells also change during the cell cycle, such that predivision cells have faster overall kinetics than post-division cells (Tamm, 1967). Are these two kinds of cell cycle changes in flagellar regeneration related? In this regard, one would like to know when in the cell cycle these changes occur, and if they are gradual or abrupt changes. A detailed analysis of the regeneration kinetics of post-division enucleate cells would not be fruitful at present, due to the small number of these cells studied so far and the greater variability in the regeneration kinetics of rotocompressor-treated cells compared with non-compressed cells.

What immediate contribution is the nucleus making to flagellar regeneration in post-division cells? It seems very likely to be RNA synthesis, from a number of experiments on the effects of enucleation on different aspects of cell metabolism (Brachet, 1961; Prescott, 1959, 1961; Mazia & Prescott, 1955; Tartar, 1961). In addition, inhibitors of RNA synthesis inhibit flagellar and ciliary regeneration to various degrees in protozoa of unknown ages (Dubnau, 1961; Child, 1965; Whitson, 1965). In *Peranema*, it will be interesting to see if a chemical inhibitor of RNA synthesis has the same effect on flagellar regeneration as enucleation.

Is the nuclear contribution to flagellar regeneration in post-division cells also essential for normal flagellar regeneration in predivision cells? If the nuclear contribution is RNA, this question could be restated: do post-division and predivision cells require *de novo* protein synthesis to the same extent for regeneration? Flagellar regeneration in non-dividing populations of *Euglena* and *Ochromonas* requires *de novo* protein synthesis (Rosenbaum & Child, 1967). One would like to know the effects of protein synthesis inhibitors on flagellar regeneration at different stages in the cell cycle.

In the preliminary experiments on increasing the enucleation-to-amputation interval, no further decline in regeneration capacity (beyond that initially observed) was found in several post-division enucleate cells when amputation was performed up to three hours after enucleation. This suggests that the decreased regeneration capacity of post-division enucleate cells whose flagella were amputated within 30 min after enucleation is not due to partial breakdown of an unstable nuclear factor during the period between enucleation and amputation. Further experiments along this line are planned.

The recent evidence for DNA in basal bodies (Randall & Disbrey, 1965; Smith-Sonneborn & Plaut, 1967) complicates the problem of flagellar development in an

exciting way, for one must now consider the possible role of basal body DNA in flagellar morphogenesis.

It is a pleasure to thank my teacher, Dr Frank M. Child, for his guidance and support in this work. I am also very grateful to Dr Joel L. Rosenbaum for his abundant enthusiasm and criticism.

This study is based on a dissertation submitted to the Zoology Department of the University of Chicago, in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

Support was provided by a Public Health Service grant GM-06879 to Dr F. M. Child; a Public Health Service training grant 2T1-GM-150; and a Public Health Service Predoctoral Fellowship, 1-F1-GM-31, 620-01, awarded to the author.

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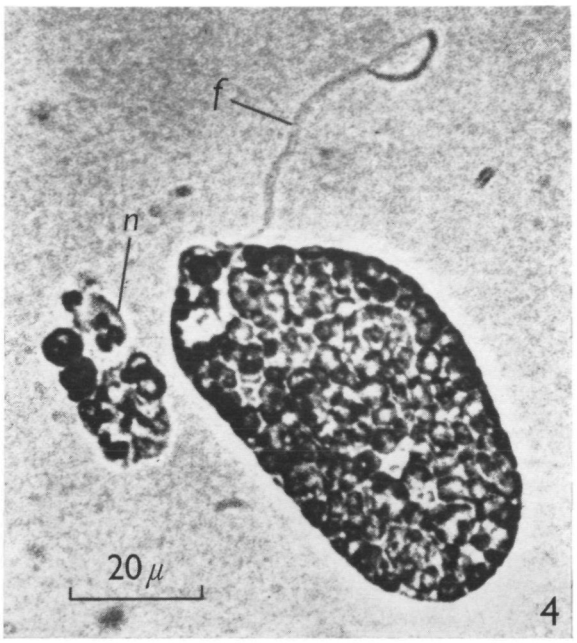
(*Received* 27 *March* 1968)



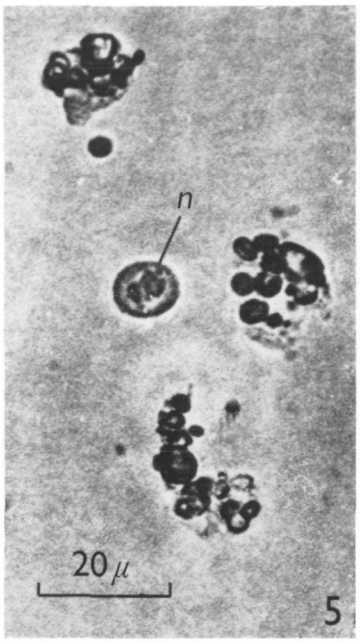




Fig. 4. Photograph of an enucleated predivision cell in the rotocompressor. The polyendosomal nucleus (*n*) has just been ejected from the cell, along with some cytoplasm. (*f*, flagellum.)

Fig. 5. Photograph of extruded cytoplasm and the nucleus (*n*) from another predivision cell in the rotocompressor. Pressure has been released and the cell has moved out of the field of view.