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Modifications of size and pattern of microtubular organelles in overfed cells of a ciliate *Dileptus*

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SUMMARY

The size of mouthparts and their constituent organelles was studied in cells of *Dileptus anser*, enlarged by overfeeding. The oral structures of large cells were either of normal dimensions and appearance, or they were enlarged and deformed in shape. The increase in size of mouthparts was accompanied by an increase in number of their microtubular organelles. There was, however, no net increase in size of the organelles, as defined by length and number of microtubules they contained.

It is concluded that there exists an upward regulation in the size of the mouthparts as a whole, whilst the size of their constituent organelles is probably not so regulated. It is supposed that the patterning of normal-size organelles into large oral structure may lead to the observed deformations in the shape of the mouthparts.

INTRODUCTION

Dileptus and many other ciliates belong to the relatively small group of organisms which maintain the properties of regulative developmental fields in their mature life (Fauré-Fremiet, 1948; Frankel, 1982). The highly organized ciliary pattern and specific forms of ciliates make possible the observations on regulatory processes that follow distortions of the equilibrium wholeness of the field. The regulatory properties of fields are expected to ensure that the proportionality of parts is maintained both in enlarged and in diminished fields.

A drastic decrease in size of a ciliate body brings about a decrease in size of the oral apparatus, which is executed either *in situ* by remodelling oral structures of *Stentor* and *Dileptus* (Tartar, 1959; Golinska & Kink, 1977), or through *de novo* formation of small mouthparts in some hypotrichous ciliates (Dembowska, 1938; Jerka-Dziadosz, 1976). This downward regulation manifests itself as a decrease in the number of complex multikinetosomal structures, and a decrease in the total number of basal bodies in *Paraurostyla* and *Tetrahymena* (Jerka-Dziadosz, 1976, 1977; Bakowska & Jerka-Dziadosz, 1980; Bakowska, Frankel & Nelsen, 1982*a*), as well as a reduction of microtubule number in some oral organelles of *Dileptus* (Golinska, 1984). The last observation indicates that developmental signals during regulation of cortical pattern may operate not only at the level of large structures and parts of organism, but also at the level of microtubular organelles.

Key words: *Dileptus anser*, microtubule, pattern, overfeeding, mouthparts.

In most ciliates there exists a species-specific size of the cell body and of oral structures. An increase in body size is not coupled with an increase in size of ciliate mouthparts. The size of the body undergoes fluctuations from division to division, while the size of oral structures remains constant (de Terra, 1969). When a cell is unusually enlarged due to the inhibition in division rate (Batson, 1983), or due to grafting together many cell bodies (Tartar, 1954), it may still produce mouthparts of normal size. There exist, however, factors that evoke the enlargement of ciliate oral apparatus. Some genic mutations are known to favour the production of exceptionally large oral structures in *Tetrahymena* (Kaczanowski, 1976; Frankel, Jenkins, Bakowska & Nelsen, 1984*a*; Frankel, Nelsen, Bakowska & Jenkins, 1984*b*). Also an increase in the size of the oral apparatus can be brought about by change in the nutritional regimen, when small prey organisms are replaced by larger ones. This has been reported for *Euplotes* (Tuffrau, 1959), for giants – cannibals of *Blepharisma* (Giese, 1938; Padmavathi, 1961) and *Stylonychia* (Giese & Alden, 1938), and the same factor seems to operate during so-called macrostomal transformation of *Tetrahymena* (Kidder, Lilly & Claff, 1940; review in Smith, 1982). The upward regulation of size of oral apparatus in these cases involves number of multikinetosomal structures, their size (i.e. a number of basal bodies) and spacing.

The object of this study was to investigate both the size of mouthparts and the number and length of microtubules in oral organelles of the unusually large cells of *Dileptus*. The enlargement was induced by excessive feeding. *Dileptus* is a very convenient material for studies on pattern regulation, because its oral apparatus is a steady-state structure, with potential for continuous addition and removal of structural subunits (Golinska & Kink, 1976; Kink, 1976; Jerka-Dziadosz & Golinska, 1977; Golinska, 1984). The oral structure of this kind was expected to enlarge freely in response to an increase in dimension of the whole cell. The results obtained in this study indicate, however, that there exists an intrinsic size limitation both in the oral structure as a whole, and in the microtubular organelles. A possible nature of this developmental control is discussed.

MATERIALS AND METHODS

Stock cultures of *Dileptus anser* were fed every other day. A food organism, *Colpidium* sp., was added to cultures in such quantity that all colpidia were eaten up during 24 h, i.e. several colpidia per one *Dileptus* cell. Further details of culture methods are described elsewhere (Golinska & Jerka-Dziadosz, 1973).

Oral organelles were studied in cells belonging to three groups: normal cells, overfed cells, and monsters. The cells termed 'normal' were those fed like stock cultures, and harvested 24 h after feeding. At that time, many were dividing and no food was left in the culture. The normal cells were in all possible stages of the cell cycle, except late fission. 'Overfed' cells were obtained by keeping ciliates continuously in the presence of an excess of food. The medium with food was renewed daily. The overfed cells were harvested after 5 to 7 days of overfeeding. Some 'monsters' could always be found in cultures of the overfed cells. They were easily recognizable due to their atypical shape. In most cases the monster was a roughly flattened cell, with or without tail and proboscis.

Measurements of the size of cells and their mouthparts were performed on preparations impregnated with protargol, after the protocol of Tuffrau (1967), slightly modified as described in a previous paper (Golinska, 1984). Since there is a shrinkage of cells during preparation, the measurements do not represent dimensions of the living cell. They allow, however, comparison of different groups of cells.

Measurements of the size of organelles were performed by counting the number of their constituent microtubules. Data concerning the size of organelles in normal cells were already published in the previous paper (Golinska, 1984). That information provided a basis for comparison of data gathered for overfed and monster cells. The organelles studied belong to more than 20 individuals in each group of cells.

Electron-microscope methods were slightly modified standard preparative methods, as described previously (Golinska, 1983). Preparations were examined in a JEM 100 B transmission electron microscope.

RESULTS

Overfeeding as a tool to enlarge cells

Enlargement of ciliate cells is usually coupled to an elongation of the interfission period. I investigated whether the overfeeding is accompanied by changes in the timing of cell cycle.

Dileptuses, when kept in constant presence of food, became larger than normal ones, and stayed large as long as food was available. The ability of overfed ciliates to divide was studied using four groups of cells: normal dileptuses, overfed cells after the first day in the surplus of food, overfed cells after 2 to 5 days of overfeeding, and overfed cells kept 24 h without food. Always 10 cells were transferred daily into a depression slide containing 1 ml of medium. The normal cells were kept in medium containing a limited number of colpidia (see Materials and Methods), which were all eaten up during 24 h. After 24 h, the *Dileptus* cells were counted. The results are summarized in Table 1. It appears that the division rate is dramatically reduced during the first day of overfeeding. This elongation of the cell cycle in the presence of a surplus of food occurs only during the first day of overfeeding, along with an increase in cell dimensions. Later on, the large overfed cells stop enlarging their bodies and attain an interfission period almost equal to that of normal cells. Moreover, the large overfed dileptuses when released from a surplus of food do not exhibit a shortening of interfission period, as do giant cells of other ciliates when a division-inhibiting factor is removed (Giese, 1938; Giese & Alden, 1938). They simply divide without food intake, whereby their size decreases. Thus the large overfed cells are capable of dividingat a perfectly normal rate. This indicates that they are in good physiological condition.

Dimensions of body and mouthparts in large and normal cells

The shape of *Dileptus* is an elongated cylinder, named the trunk, tapered at its hind end into a slender tail. At the anterior end the ventral portion of the trunk is closed by an oral structure. The dorsal portion of trunk forms a very long process, termed the proboscis. To estimate the size of a cell body, the circumference of the trunk was chosen. Preliminary observations showed that the length of the cell

Table 1. *Division capacity in overfed cells*

Group of cells	Number of divisions per cell per day	Number of cells
Normal cells	1·07	140
Overfed cells first day of overfeeding	0·05	200
Overfed cells 2nd to 5th day of overfeeding	1·02	400
Overfed cells first day without food	0·87	200

is a useless feature for this study, because it varies within a very wide range accordingly to the stage in cell cycle, moreover, the mean length is practically the same for normal and overfed cells (Drzewinska, in preparation). The circumference of the trunk was obtained by measuring the width and depth of body in the middle of the trunk. Since on permanent preparations cells are rarely circular in cross section, the circumference was calculated using a formula for the circumference of an ellipse.

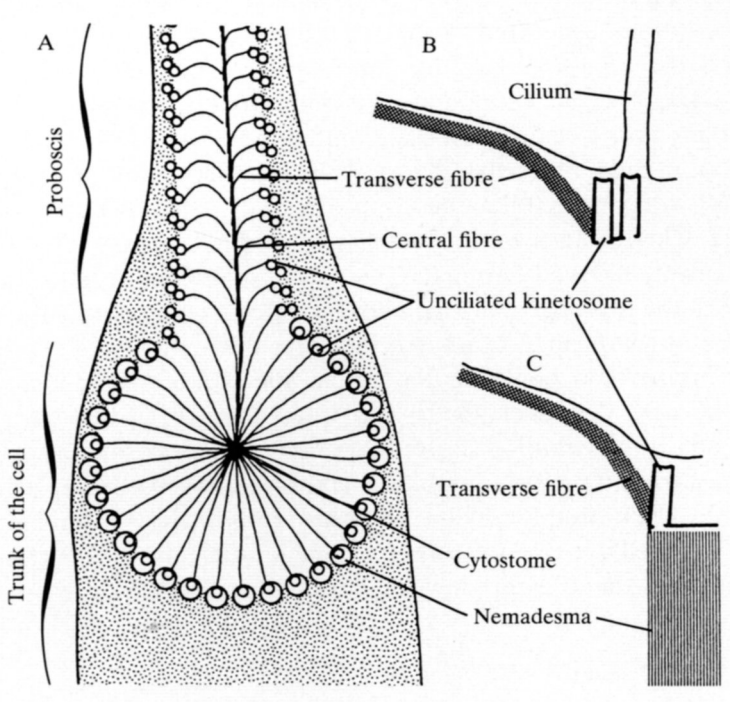
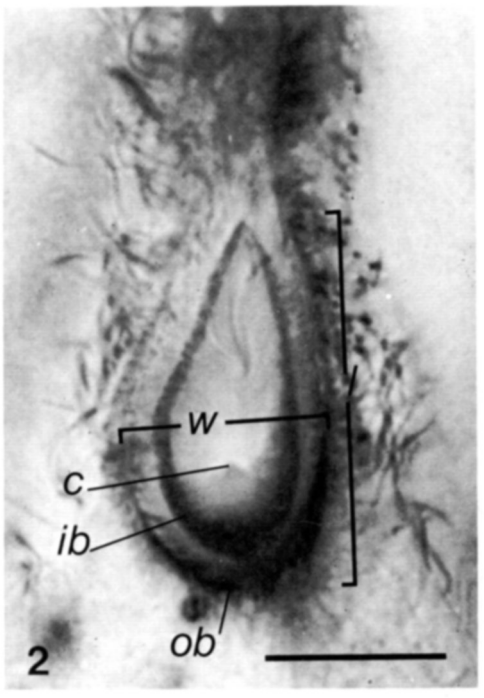


Fig. 1. (A) Ventral view of *Dileptus*. The dotted area represents the somatic region of the cell. The circular portion of the cytostomal field is situated on the trunk of cell, while the extension of the field, called the ventral band, is situated on the proboscis. (B) Side view of an oral kinetid from the margin of the ventral band. (C) Side view of an oral kinetid from the margin of the cytostomal field.

Oral structures of *Dileptus* consist of the cytostome encircled by a non-ciliated area called the oral field. At the base of the proboscis the circular portion of the oral field is situated, with the cytostome in the middle, and this is named the cytostomal field. An extension of the oral field, the so-called ventral band, occupies the ventral side of the proboscis. The margin of the cytostomal field together with the ventral band consists of a single row of oral kinetosomes. On protargol-stained preparations this row looks like a dark line (Fig. 2). Another dark line around the cytostome inside the territory of the cytostomal field represents an inner basket of microtubular bundles (details of the structure in Golinska, 1978). To estimate the size of mouthparts, the circumference of the cytostomal field was chosen, its length and width measured, then a calculation was performed similar to the calculation of the circumference of the trunk. The right and left margin of the field in its widest part served as structural markers to estimate the width of the field. The length of the cytostomal field was measured from the posteriormost part of its margin, to the anteriormost part of the inner basket (Figs 2, 3). Measurements were performed on three groups of cells (Table 2). The first group contained normal cells. These have a slender trunk and roughly circular cytostomal field (length greater than width in 52 % cells, and width greater than length in 48 % of cells, n = 50). Cells belonging to two groups of overfed cells have trunks significantly larger than normal cells. The group *overfed I* contains cells with a smooth outline of cytostomal field, while the cells belonging to the group *overfed II* have one or two indentations in the margin of cytostomal field. The group *overfed I* has a circumference of the cytostomal field that is practically the same as that found in normal cells. The cytostomal fields of normal and overfed cells are, however, different in shape. In *overfed I* cells a short and wide field was observed only in 12 % of the cells, and 88 % had a long and narrow field (Fig. 3). The maximum length was 43·5 μm. This elongation of the cytostomal field in overfed cells seems to be the first step towards the formation of a monstrous mouth. The next step is the appearance of one or more indentations in a cytostomal field (Fig. 4). Cells in the group *overfed II*, with indented cytostomal fields, have trunks of the same size as cells in the group *overfed I*. Cytostomal fields with indentations are significantly larger than those with a smooth outline, although both are situated on cells of approximately the same size. The formation of indentations is associated with the enlargement of cytostomal fields. The width and length of indented fields were measured as for fields without indentations (the additional borderline of an indentation has not been taken into consideration).

A further step in the deformation of mouthparts in overfed cells is a deepening of indentations and subsequent formation of two or more subfields (Fig. 5), each with its own cytostomal depression, which usually are joined to the same ventral band. Two-cytostomal cells are able to divide, and able to swallow their prey with at least one of cytostomes. Most of them have trunks of similar size and shape to any other overfed cell.

The next stage of structural disturbance in mouthparts of overfed cells is a further multiplication of cytostomal subfields, accompanied by gradual loss of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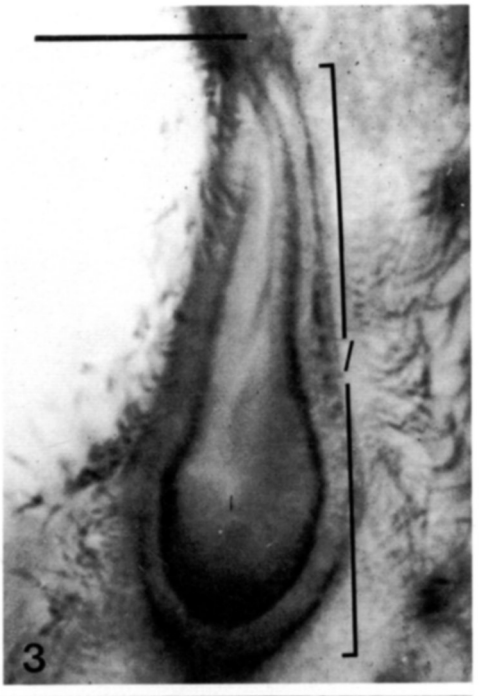
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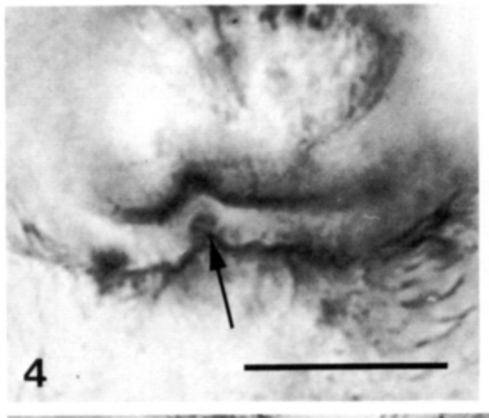
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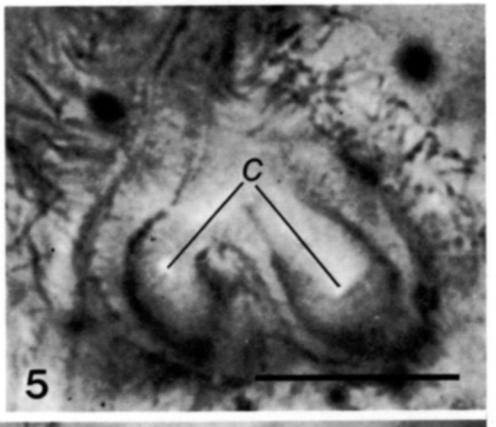
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Table 2. *Sizes of cell body and mouthparts in the overfed and normal cells*

Group of cells	Circumference of cytostomal field (μm)	Circumference of trunk
Normal n = 50	69·6 ± 9·2	139·7 ± 18·3
Overfed 1	68·3 ± 9·7	215·5 ± 32·9
Overfed 2	80·4 ± 12·6	203·0 ± 36·1

Values are given ±S.D. The broken line separates the values that differ significantly from each other.

regular shape of the cell. The resulting monster is unable to divide or feed. It is a roughly circular flattened mass with a fantastic decoration of wildly meandering oral structures (Fig. 6). The size of each individual subfield is always inferior to the size of the cytostomal field in a normal cell, but the total area of cytostomal fields is much greater than normal. The increasing monstrosity of the overfed cells proceeds alongside with a shortening or pinching off of their probosces. It has not been studied whether or not the monsters are able to reorganize themselves into normal cells.

Relationship between the size of cytostomal field and the number of organelles at its circumference

The question whether an increase in the size of cytostomal field is accompanied by an increase in the number of oral kinetosomes at its circumference, cannot be answered directly by counting the kinetosomes. In light microscope preparations these basal bodies, together with their fibrillar associates, appear as an almost continuous line. Only a small portion of the margin of cytostomal field can be seen on electron microscope sections that are suitable for counting. It is the density of oral kinetosomes in a row at the margin of cytostomal field that can be compared in normal and overfed cells, using electron micrographs.

To estimate the crowding of kinetosomes, the ratio of the length of segment covered by ten successive kinetosomes, to the outer diameter of a kinetosome was

Figs 2–6. Cytostomal fields of normal and overfed cells. Protargol-stained preparations. Bar, 50 μm.

Fig. 2. The cytostomal field of a normal cell. *c*, cytostome; *ib*, internal basket; *ob*, outer basket of nemadesmata, *w* and *l* show how the width and length of fields was measured.

Fig. 3. Elongated cytostomal field in a cell after 5 days of overfeeding. *l*, length of the field.

Fig. 4. The indentation in the cytostomal field after 5 days of overfeeding is indicated by arrow.

Fig. 5. A cytostomal field subdivided into two subfields with two cytostomes (*c*). 5 days of overfeeding.

Fig. 6. Oral structures on the surface of a monster. 9 days of overfeeding.

chosen. In 19 normal cells this ratio ranged from 12 to 19 (mean 15·2 ± S.D. 2·1), and in 15 overfed cells the ratio varied from 12 to 20·4 (mean 14·5 ± S.D. 2·3). Comparison of means (Student *t* test) indicates that the differences between means are non-significant, and oral kinetosomes around the cytostomal field of normal size are as densely packed as kinetosomes around the enlarged cytostomal field. Thus the enlarged mouthparts, having the longer outline, contain an increased number of oral kinetosomes.

Size of microtubular organelles in normal and overfed cells

Measurements of the size of microtubular organelles were performed in normal and overfed cells in order to check whether an increase in the dimensions of the cell is reflected in the size of organelles. The organelles studied were, as in a previous study (Golinska, 1984), nemadesmata and transverse fibres. The organelles are the fibrillar associates of non-ciliated oral kinetosomes. The oral kinetosomes form a single row at the circumference of the oral field, and each kinetosome gives rise to one transverse fibre and to one nemadesma (Fig. 1). Nemadesmata extend deeply into the endoplasm, forming the palisade around the cytostome called the pharyngeal basket. Transverse fibres are ribbons of microtubules running under the surface of the oral field.

(A) *Nemadesmata*

The nemadesma is a bundle of hexagonally packed microtubules. Its top is closed with two layers of dense material separated by a clear zone (Grain, 1969). In *Dileptus* the upper dense layer, called the upper sheet (terminology after Pearson & Tucker, 1977), consists of very dense amorphous matter in which the proximal part of the oral kinetosome is embedded (Figs 7, 8, 10). The upper sheet does not close the proximal end of the kinetosome, so that the lumen of basal body

Figs 7–12. Nemadesmata in normal and overfed cells. The bar represents 0·5 μm. *n*, nemadesma; *k*, kinetosome; *tf*, transverse fibre; *d*, dense upper sheet of nemadesma; *p*, perforated sheet.

Fig. 7. An oral kinetid on the margin of the cytostomal field in a normal cell. In this small nemadesma the upper sheet is of the same size as perforated sheet. The lumen of the kinetosome opens into a clear space between the upper and perforated sheets of nemadesma.

Fig. 8. An oral kinetid on the margin of the cytostomal field in an overfed cell (7 days of overfeeding). This large nemadesma has an upper sheet much smaller than the perforated sheet.

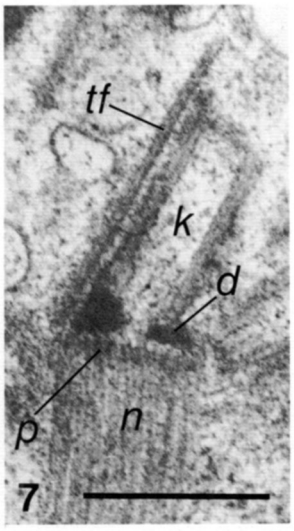
Fig. 9. The margin of a cytostomal field after 5 days of overfeeding. *m.f.*, microfibrillar layer at the level of proximal ends of kinetosomes. The arrow shows nemadesma below this level, separated from its kinetosome, and topped with only the perforated sheet.

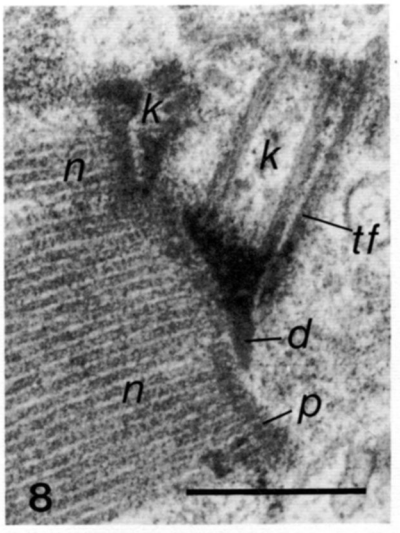
Fig. 10. Grazing section of tops of nemadesmata in an overfed cell (7 days of overfeeding).

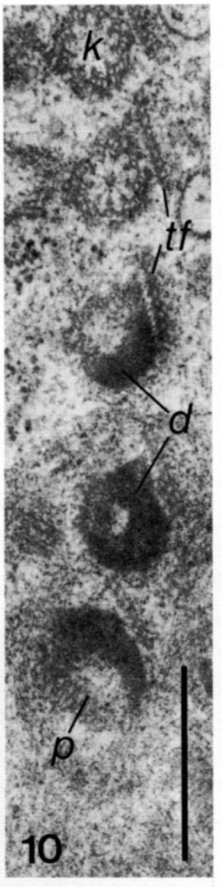
Fig. 11. The top of a nemadesma separated from its kinetosome, showing an incomplete perforated sheet (*p*). 7 days of overfeeding.

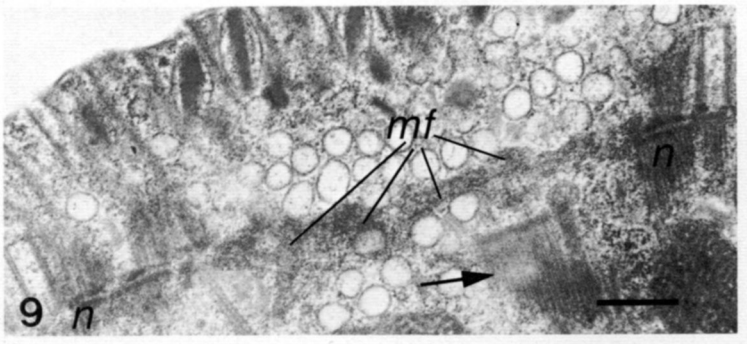
Fig. 12. The top of a disintegrating nemadesma with some microtubules missing and an incomplete perforated sheet (*p*).

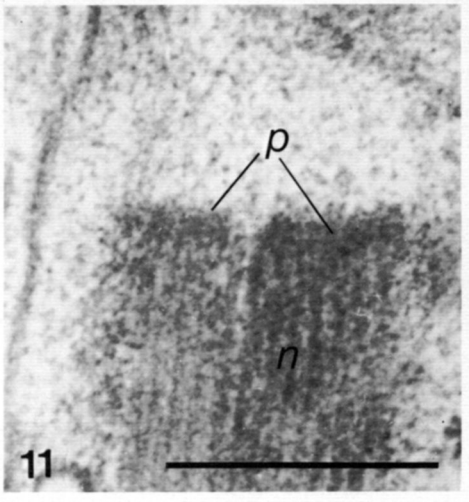
and a clear space between the two dense layers are in direct continuity (Figs 7, 10). The other dense layer is called the perforated sheet, and consists of tips of microtubules and fibrogranular material in which the tips are embedded. Both

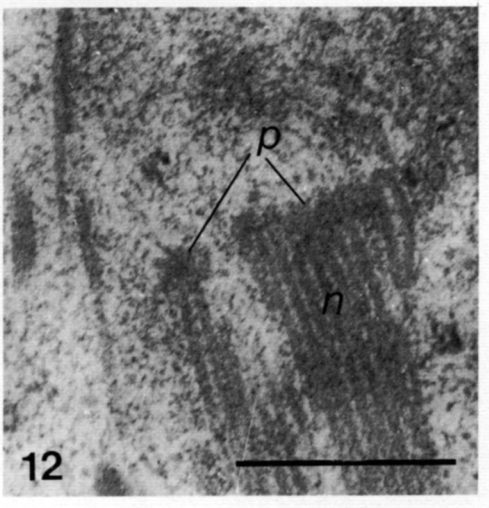












dense layers are interconnected with short links. With large nemadesma the upper sheet is much smaller than the perforated sheet (Fig. 8). From this three-layered cap extends the rod of hexagonally packed microtubules held together by numerous intertubular links.

The size of nemadesmata was estimated by counting their constituent microtubules. In this study, the nemadesmata originating from three cell groups were compared. One group consisted of overfed cells that retained normal shape after 5 days of overfeeding. In this group, the nemadesmata originated from normal, elongated, indented or subdivided cytostomal fields. The second group consisted of monsters picked up from cultures of overfed cells after 7 to 9 days of overfeeding, thus their nemadesmata all belonged to baskets subdivided into multiple structures. The third group consisted of normal cells, the same data as used in the previous paper (table 1 and fig. 5 in Golinska, 1984).

There are several features common for nemadesmata from all three groups. The size of nemadesmata varies within a very wide range (Table 3, Fig. 13). Moreover, the small and large organelles are mixed together, so that there is no part of a basket with uniform nemadesmata. In all groups, the smallest organelles contain only several microtubules.

The mean number of microtubules in nemadesmata from overfed cells does not differ significantly from the mean found for nemadesmata of monsters (Mann-Whitney U test). Thus further analysis was performed only on two groups of nemadesmata: those from overfed and normal cells, the nemadesmata from monsters treated as a part of the overfed group. There are differences between the two groups of nemadesmata in their mean microtubule content, and in the frequency distribution of their microtubule number. The mean number of microtubules in the nemadesmata from overfed cells is significantly lower than the mean in normal cells (Mann-Whitney U test, $P < 0{\cdot}001$). The most severe reduction is in maximum size of nemadesma: from 448 microtubules in normal cells to 244 in the overfed ones (Fig. 13, Table 3). This reduction in quantity of large organelles is not compensated for by appropriate changes in quantity of smaller organelles, since frequency distributions of microtubule content in both groups are significantly different (Smirnov test, $P < 0{\cdot}05$). In both normal and overfed cells the frequency distribution is asymmetrical, drawn out in the direction of small nemadesmata (Fig. 13). This indicates that new small nemadesmata are formed both in normal and overfed cells. The differences in frequency distribution indicate that the groups of cells differ in the rate of growth and/or destruction of these organelles.

The destruction of nemadesmata has never been observed in normal cells. This does not necessarily mean that nemadesmata are never destroyed in normal cells but suggests that destruction is rare. In overfed cells and in monsters electron microscope images were often encountered which indicate that some nemadesmata have detached from their kinetosomes (Fig. 9), and were undergoing resorption (Figs 11, 12). Nemadesmata are seen in different stages of destruction, with their capped ends situated well below the level where other nemadesmata are

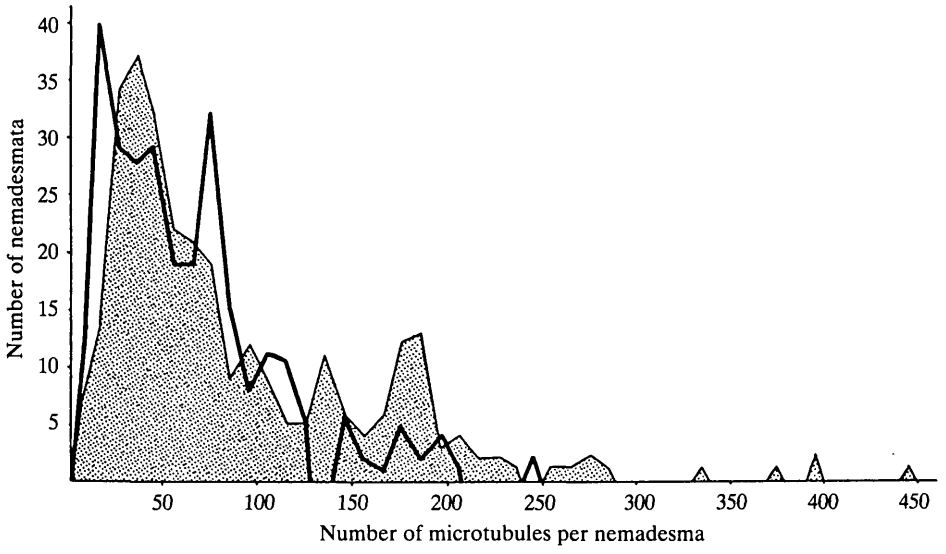


Fig. 13. Number of microtubules in nemadesmata of normal cells (dotted area) and collective group of overfed and monster cells (heavy line).

linked to oral kinetosomes (Fig. 10). The first stage of destruction appears to be the loss of connection with the kinetosome, and the disappearance of the upper sheet of the cap. In later stages, nemadesmata move down the basket along neighbouring nemadesmata. The remnant of the cap, the perforated sheet, becomes more and more incomplete (Figs 11, 12), and the number of microtubules gets smaller.

It is not clear what happens to oral kinetosomes after their nemadesmata have detached. The kinetosomes were never seen below the microfibrillar layer, where their proximal ends are usually anchored. There are several possibilities. Oral kinetosomes can be resorbed *in situ*, before or after their nemadesmata move deeper into the endoplasm. They can be preserved, retaining some portion of the detached nemadesma, or can form a new organelle in place of the lost one. This could represent an additional source of small nemadesmata, in addition to those formed by proliferation at the circumference of the cytostomal field. This latter mechanism occurs in all three groups of cells.

Table 3. *Number of microtubules in nemadesmata from three groups of cells*

Group of cells	Range of microtubule numbers	Characteristics of frequency distribution			No. of nemadesmata analysed
		Mean	Mode	Median	
Normal	7–448	86	35	60	300
Overfed	4–244	64	20	60	240
Monsters	10–121	62	75	70	41

Table 4. *Number of microtubules in transverse fibres and number of left transverse fibres in central fibre*

Group of cells	No. of microtubules in transverse fibre			No. of left transverse fibres in central fibre
	Left transverse fibre	Right transverse fibre	Cytostomal transverse fibre	
Normal*	25·8 ± 2·0 range 20–31 n = 140	15·2 ± 1·1 range 12–18 n = 125	16·5 ± 1·9 range 14–23 n = 101	12·4 ± 2·0 range 7–16 n = 96
Overfed	24·2 ± 5·1 range 7–38 n = 91	15·0 ± 1·8 range 5–18 n = 82	15·7 ± 2·3 range 8–19 n = 87	12·4 ± 2·0 range 9–16 n = 23
Monsters	24·0 ± 7·2 range 3–37 n = 87	14·4 ± 4·1 range 3–20 n = 55	no data	12·6 ± 2·6 range 9–18 n = 16

* Data taken from Golinska (1984, table 2).

The relatively small size of nemadesmata in overfed and monster cells, and frequency with which images suggesting destruction are encountered, indicate that the lifetime of nemadesmata can be drastically shortened as a consequence of overfeeding.

(B) *Transverse fibres*

In *Dileptus* each mature and unciliated oral kinetosome gives rise to a ribbon of microtubules perpendicular to the surface of the cell. These ribbons, transverse fibres, are arranged in a characteristic pattern on the surface of the oral field (Fig. 1). On the cytostomal field all ribbons run toward the cytostome, and some of them enter a cytostomal depression. The transverse fibres coming from the left and right sides of the ventral band run a short way toward the middle of the band, then curve and run posteriorly, in the direction of the cytostome. The curved parts of the left transverse fibres form a bundle in the territory of the ventral band, the so-called central fibre (Figs 1, 15). The number of microtubules in a transverse fibre depends on the part of the oral field that the fibre occupies. The largest ribbons were encountered on the left side of the ventral band, while those on the right side of the band are much smaller, and similar in size to ribbons on the cytostomal field. In this study, as in a previous one (Golinska, 1984), the number of microtubules was counted in ribbons originating from three territories: the cytostomal field and both sides of the ventral band in the proximal part of proboscis.

The results of microtubule counting are summarized in Table 4. The mean number of microtubules in transverse fibres is practically the same in normal, overfed and monster cells, when analysed using Kruskal-Wallis test for left transverse fibre ($P = 0{\cdot}12$) and right transverse fibre ($P = 0{\cdot}56$). The mean transverse fibres on cytostomal fields of normal and overfed cells are slightly

different (Mann-Whitney U test, $P < 0{\cdot}05$). Variability of microtubule number in the ribbons is, however, much increased in overfed and monster cells when compared to normal ones. This is indicated by the *f* test of variance ratios, and also by comparison of the range of microtubule number in different groups of cells (Table 4, Fig. 14). The range of the left and right transverse fibres is widened in both directions: ribbons containing more as well as less microtubules than ribbons in normal cells can be found in overfed and monster cells. On cytostomal fields of overfed cells transverse fibres containing more microtubules than those of normal cells were not found, and the range for transverse fibres from this region is shifted only in the direction of small ribbons. No data are available for the number of microtubules in transverse fibres from the cytostomal field of monsters. Almost all of the oral structures found on the surface of a monster, when examined in the electron microscope, reveal a fine structure characteristic of the ventral band.

Unusually large ribbons were mainly encountered on the left side of the ventral band. The largest ribbon found in an overfed cell contained 38 microtubules, i.e. 7 microtubules more than the largest left transverse fibre found in a normal cell. Such exceptionally large ribbons are only rarely encountered (Fig. 14). Unusually small ribbons can often be observed both in the overfed cells and in monsters.

The pictures obtained in the electron microscope help to explain the low number of large ribbons and high number of small ribbons in overfed cells and in monsters. The ribbons of transverse fibres were observed to bend and split

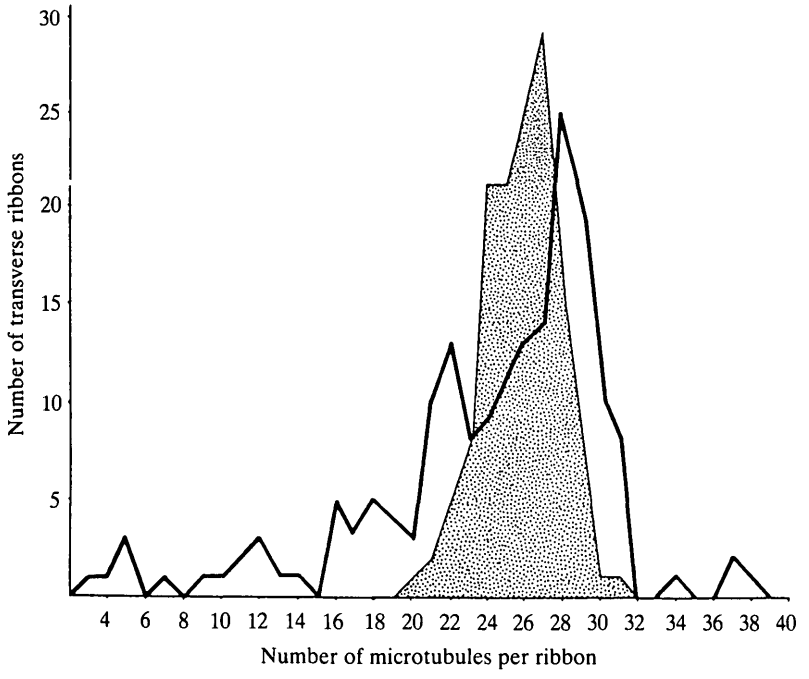


Fig. 14. Number of microtubules in left transverse fibres of normal cells (dotted area) and collective group of overfed and monster cells (heavy line).

longitudinally (Figs 16, 18), the portion of the ribbon separated from the cell surface may be resorbed later. The images of splitting ribbons were seen in all parts of the oral field, but the most spectacular images were found in the central fibre, where many left transverse fibres are gathered together. The central fibre in a normal cell consists of ribbons of almost the same size (Fig. 15). In an overfed cell it contains ribbons that are bent, disrupted, or very small between those of normal appearance (Fig. 16). Amongst 25 central fibres found on appropriate transverse sections in overfed cells, only 6 contained transverse ribbons composed of almost the same number of microtubules. The remaining 19 central fibres contained ribbons of unequal size. In addition to bending and splitting, the ribbons found in central fibres of monster cells can also show abnormal spacing of ribbons (Fig. 17) and may be detached from the cell surface (Fig. 18). The unusually small ribbons in transverse fibres of the overfed and monster cells can be explained as a reduction in microtubule number through longitudinal splitting of ribbons. No data are available on whether the splitting of a ribbon occurs on its whole length, or whether it takes place only in its distal part, with the proximal part left intact, with its MTOC portion attached to an oral kinetosome.

Estimation of the length of microtubules in left transverse fibres

The fact that left transverse fibres are gathered up into a central fibre presents an opportunity to estimate the length of their microtubules. The central fibre consists of distal parts of left transverse fibres, which are arranged into closely apposed parallel ribbons (Fig. 15). This tight packing of ribbons allows us to distinguish in a cross section the left transverse fibre belonging to central fibre from its neighbours. Each ribbon is linked to a kinetosomal pair of the left margin of the ventral band (Fig. 1). The number of ribbons in the central fibre reflects the number of kinetosomal pairs linked to these ribbons. Preliminary observations showed that the distances between kinetosomal pairs on the left margin of the ventral band are the same in normal cells and in overfed ones, while in monsters the kinetosomal pairs are slightly more crowded. We can estimate the length of transverse fibre microtubules. The higher the number of the transverse fibres in the central fibre, the longer are microtubules in these ribbons. When ribbons in central fibres of overfed and monster cells were counted, only the ribbons which retained contact with a cell surface were taken into consideration to avoid double counting of split ribbons. Also the central fibres with irregularly spaced ribbons, found in some monster cells (Figs 17, 18), were rejected. Thus only the ribbons closely apposed and linked to the cell surface were counted as belonging to the central fibre.

The number of ribbons in the central fibre was found to be the same for all three groups of cells (Table 4). The means do not differ significantly when compared using Kruskal-Wallis test ($P = 0{\cdot}95$), and f test shows that the variances are the same. The range of variability in the number of ribbons per central fibre is not greater in overfed cells than in normal ones. One unusually large central fibre has been found in a monster, and this contained only two ribbons more than the maximum number (16) of ribbons encountered both in normal and overfed cells.

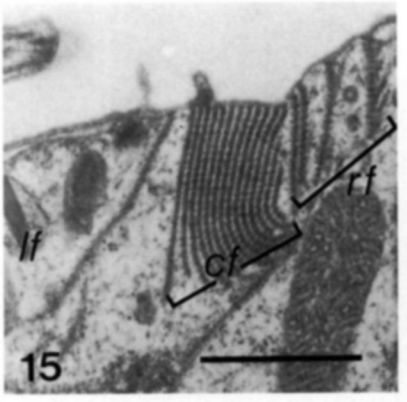
This does not mean that this large fibre was built up of especially long ribbons, since monsters manifest an increased crowding of kinetosomal pairs on the left margin of the ventral band.

Thus there is neither an increase nor a decrease in the length of left transverse fibres in the overfed cells, in spite of the increased variability in the number of microtubules they contain.

DISCUSSION

The results can be summarized as follows: the enlarged mouthparts in overfed cells of *Dileptus* have lengthened margins of the cytostomal field, and the oral kinetosomes situated at this margin form an array of the same density as observed in normal cells. This indicates that during the upward regulation of dimensions of oral apparatus there is an increase in number of oral kinetosomes and their fibrillar associates, namely nemadesmata and transverse fibres. There is, however, no net increase in size of these microtubular organelles. Unusually high numbers of microtubules can be found only in a few transverse fibres. The majority of microtubular organelles in an enlarged oral structure contain fewer microtubules than those in the mouthparts of normal size. The length of microtubules in transverse fibres, at least in the left transverse fibres, is the same in normal and overfed cells.

An increase in size of cytostomal field of *Dileptus* is accompanied by deformations of its shape. The enlargement of oral structures in *Tetrahymena* (Smith, 1982; Frankel *et al.* 1984*a,b*) and *Paraurostyla* (Bakowska & Jerka-Dziadosz, 1980) is coupled with deformations in shape of large multikinetosomal complexes termed the membranelles. The method of development of membranelles causes their widths to remain the same, while the lengths of membranelles increase with increased number of oral kinetosomes, resulting in a change of internal proportions of the membranelles. The deformations of large cytostomal fields in *Dileptus* could also be ascribed to the ways of patterning of this structure. A cytostomal field grows by addition of new oral kinetosomes at its circumference, and their subsequent insertion in between preexisting organelles (Kink, 1976; Golinska & Kink, 1976). The transverse fibres associated with these kinetosomes are all directed toward the cytostome and many of them enter the cytostomal depression. The sculpturing and maintenance of the cytostomal depression may depend upon the presence of distal parts of transverse ribbons coming from different directions. When the cytostomal field enlarges, due to the increased number of kinetosomal elements on its circumference, the only way to maintain the circular shape of the cytostomal field would be the elongation of transverse fibres. If the length of ribbons on cytostomal field is invariant, as that of left transverse fibres is, the only way to allow the distal ends of transverse fibres to reach the cytostome would be the elongation, folding, and subdivision of cytostomal fields, i.e. exactly these deformations that were observed in the overfed cells.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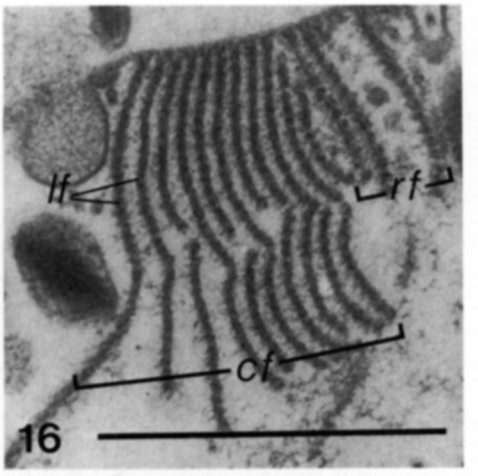
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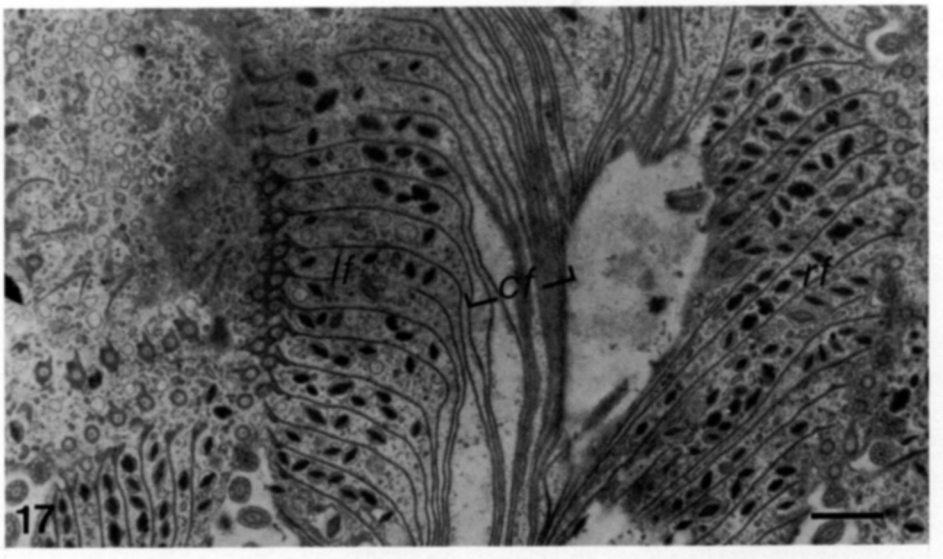
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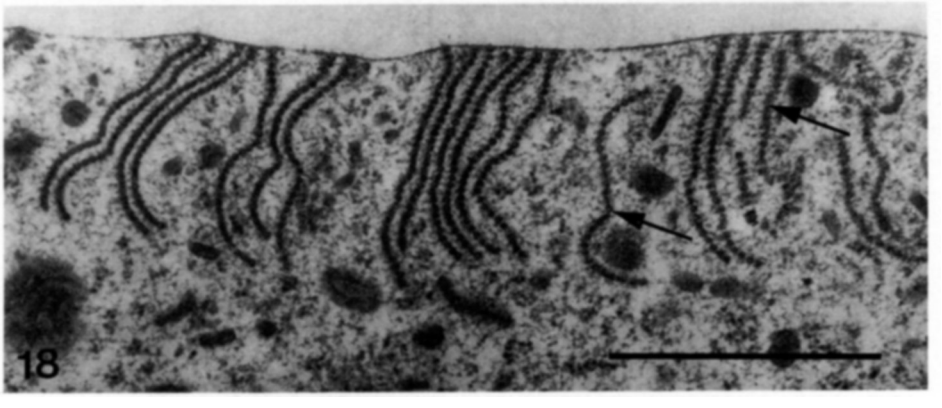
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The inability of transverse fibres to grow above their normal length is especially interesting when substantial reduction in length of these fibres in miniaturized cells is recalled (Golinska, 1984). Several mechanisms which might control microtubule length have been proposed in other cell types (Roth, Pihlaja & Shigenaka, 1970; Brinkley *et al.* 1981; Tucker, 1982, for review see Aufderheide, Frankel & Williams, 1980). I have no evidence to suggest which applies in this case, but for *Dileptus* the same mechanism may control both the size of oral structure and the length of microtubules in transverse fibres. It is not known whether such a mechanism operates during development of mouthparts of other ciliates, but a similar mechanism has been proposed for determination of size of cortical territories (Pitelka, 1969).

The enlargement of the cell body in *Dileptus* is not necessarily followed by the enlargement of mouthparts, since numerous large overfed cells were found to possess the cytostomal field of normal size. What keeps the size of mouthparts down is probably the mechanism controlling the rates of proliferation and destruction of oral organelles. It can be imagined that the rates of both processes in enlarged cells with normal-sized mouthparts are either maintained at the normal level a long time after the enlargement of the cell, or that each change in the rate of proliferation is balanced by a similar change in the rate of destruction of organelles. Such a conception of the control mechanism as a simple resultant of the proliferation–resorption processes, is certainly the far-fetched simplification. It allows, however, comparison of data known for very different organisms. In other than *Dileptus* ciliates, the size of mouthparts is determined during a short period of its development, but it also involves both formation and destruction of oral organelles. In *Condylostoma* (Bohatier, Tuffrau & Tuffrau, 1976), *Paraurostyla* (Jerka-Dziadosz, 1981) and *Tetrahymena* (Bakowska, Nelsen & Frankel, 1982*b*), an intense proliferation of oral kinetosomes in the early stage of oral development is followed by a simultaneous resorption of some of the newly formed kinetosomes and a second round of kinetosome proliferation.

During the formation of abnormally large mouthparts in some mutants of *Tetrahymena thermophila* (Frankel *et al.* 1984*a,b*), there is not only a higher production of oral kinetosomes, but also the destruction is increased. The same is probably true for the large mouthparts of overfed dilpetuses, because the images of resorption are unusually frequent in these cells. This increased destruction does not, however, equal the increased proliferation and large mouthparts are forming.

Figs 15–18. Transverse fibres in normal and overfed cells. The bar represents 1 μm. *cf*, central fibre; *rf*, right transverse fibre; *lf*, left transverse fibre.

Fig. 15. A central fibre in normal cell.

Fig. 16. A central fibre in a cell after 7 days of overfeeding. Note the bent and disrupted ribbons.

Fig. 17. A portion of the oral structures of a monster after 9 days of overfeeding. Note the irregular spacing of left transverse fibres in place of a central fibre.

Fig. 18. A cross section of the irregularly spaced left transverse fibres in a monster (9 days of overfeeding). Some of the ribbons are separated from the cell surface (arrows).

When this happens in oral structures that can grow only during the period of their development, like in *Tetrahymena*, the size of mouthparts may be additionally limited by the time of their formation. In *Dileptus*, once the balance between proliferation and destruction is lost, it may lead from the continuous exchange of oral organelles to their continuous increase in number, and thereby to formation of uncontrollably growing mouthparts. It seems that the restriction of organelle formation to the time of development of oral structure is the important step in evolution of compounded oral apparatuses in ciliates.

The enlarged oral structures of *Dileptus* comprise organelles which only rarely contain an increased number of microtubules. This may be caused by a structural instability of microtubular organelles in overfed cells. The nemadesmata were observed to detach from their kinetosomes and disintegrate. The transverse fibres undergo longitudinal splitting, and resorption of the separated portion. This instability does not allow clarification of the important question, of whether the microtubular organelles have the capacity to regulate their size according to the increased size of the cell. The size of organelles can be defined by the length and number of microtubules they contain. It has been found in a previous study (Golinska, 1984) that nemadesmata of *Dileptus* may grow continuously, adding new microtubules laterally to already existing ones, while the transverse fibres once formed do not enlarge further by addition of new microtubules. Thus the reduced microtubule number in average nemadesma in the overfed cells may be simply a result of shortening the lifespan of microtubular organelles, and nemadesmata probably do not persist long enough to attain large dimensions. The transverse fibres in overfed cells show strikingly increased variability in their microtubular number, even among fibres in one region of the same cell. Fibres containing more microtubules than have been encountered in normal cells were sometimes observed, in conjunction with smaller fibres. Since some portions of transverse fibres can be lost by splitting, the formation of unusually large ribbons can be more frequent than is indicated by their observed quantity in overfed cells. Nonetheless, most of the transverse fibres found in overfed and monster cells fall within the size range of normal ribbons. This indicates that besides unusually large ribbons, the overfed cells also produce those of normal size, and the formation of small ribbons also cannot be excluded. If the formation of an especially large ribbon were the response of its MTOC to the increased size of the cell, it would be hard to understand why the MTOCs of neighbour transverse fibres do not form ribbons of similar size. During the downward regulation of microtubule number in miniaturized cells (Golinska, 1984), the decrease in microtubule number has been found to be very similar for all ribbons within a region. Thus it seems that the formation of unusually large ribbons in overfed cell may rather be the result of the less-precise control over the microtubule number its MTOC can nucleate, than the response to a developmental signal concerning increased size of the cell.

It is concluded that there exists an upward regulation in the size of the mouthparts and in the number of their constituent organelles, while the size of these organelles is probably not so regulated. Patterning of the normal-size

organelles into large-size mouthparts leads to deformations and monstrosities of oral structures. This indicates that the limited size of the microtubular organelles is the factor which can control the upper size of oral structure, and thereby the size of the whole cell.

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