

A LOCALIZED REGION OF BASAL BODY
PROLIFERATION IN GROWING CELLS OF
DILEPTUS VISSCHERI (CILIATA,
GYMNOSTOMATA)

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SUMMARY

In the ciliate *Dileptus* organisms are completely organized at the time of excystation. They grow intensively, increasing in volume several times, before they reach the trophic cell size. The growth includes both the somatic part of the body as well as the oral parts, consisting of the cytostomal field and the ventral band of the proboscis. From the time of excystation, during the growth of the cell, the oral apparatus is able to capture and ingest food. Growth of an oral apparatus while functioning has not previously been reported in ciliates.

The proliferation of new oral kinetosomes in the growing oral apparatus occurs only in the cytostomal field. There is no proliferation of ciliature in the oral kineties of the growing proboscis. In those regions of the somatic kineties bordering the cytostomal field an extensive proliferation of somatic ciliature takes place. The zone of proliferation common to the oral and somatic ciliature supplies new kinetosomes for the elongating proboscis, the growing oral apparatus and the growth of the rest of the body.

The mode of proliferation of the oral kinetosomes differs from that of the somatic kinetosomes. The oral kinetosomes form randomly in large numbers, appearing similar to an anarchic field such as is found in other ciliates. The somatic kinetosomes always arise singly and in a defined position in relation to the mature kinetosome.

The proliferation of basal bodies in a defined area in *Dileptus* suggests the existence of a growth zone localized around the cytostomal field.

INTRODUCTION

In ciliates thus far studied it has been well documented (Schwartz, 1935; Buhse, 1966; Tartar, 1967; Buhse & Corliss, 1969; de Terra, 1969) that the oral apparatus can form and grow only during the process of stomatogenesis. In the present study we deal with a rapidly growing, but already completely developed organism – the newly excysted cell of *Dileptus vissscheri*. Preliminary observations indicate that after escaping from the cyst wall the ciliate doubles in size before it reaches the size characteristic of a normal trophic cell of this species. There is growth of somatic as well as oral parts of the body. The oral structures are fully differentiated and capable of capturing and ingesting food from the moment that the ciliate leaves the cyst wall. In these excysted cells growth proceeds in the absence of stomatogenesis.

There are two possibilities as to the way that this growth proceeds. It may be due either to an enlargement of spaces between old kinetosomes, or to formation of new

kinetosomes. In this study evidence is presented that there is extensive proliferation of kinetosomes in both somatic and oral regions. The pattern of the new kinetosomes intruded into the cell surface during growth of oral and somatic parts is described.

MATERIAL AND METHODS

Dileptus vissscheri was cultivated in beakers. Pringsheim solution was used as a medium. Food was supplied every day in the form of a washed and concentrated mass culture of *Colpidium*. In cultures which were extensively fed without renewing the culture medium, mass encystment occurred (Kink, 1973). The cysts were kept in the same dishes and the medium was occasionally renewed by adding fresh Pringsheim solution. After 3–4 weeks excystment was observed. This process lasted 2–3 days; the ciliates escaped from the cyst walls within 12–15 h after the first movements inside the cyst body were observable. Excystment was observed in less than half of the sample of synchronously encysted cells. The remaining cysts did not excyst even during the subsequent 2–3 months. It seems that most of them were dead.

Excysted individuals grew and divided, forming normal populations. Non-fed excysted animals were also able to grow and to divide. The animals investigated in this study were fixed 0–12 h after escape from the cyst walls.

For light-microscope observations, the animals were fixed in Hollande fluid and stained with Protargol by the method of Tuffrau (1964), using Javel water after fixation and gold chloride for contrasting. For electron microscopy, cells were fixed in fresh 2% OsO₄ or in a mixture of 2% OsO₄ and 6% glutaraldehyde dissolved in cacodylate buffer. The cells were kept on ice. After washing the cells were grouped in 0.5% agar and embedded in Epon following dehydration. The material was sectioned on a Tesla ultramicrotome. The sections were stained with uranyl acetate and lead citrate and examined with a JEM 100B.

RESULTS

The ciliate *Dileptus* has an elongated body terminating in a tiny tail at the posterior pole. At the anterior pole there is a cytostome encircled by the cytostomal field, bordered with the oral ciliature. The cytostomal field is elongated on the ventral side of the proboscis, where it forms the so-called ventral band.

The dorsal part of the proboscis consists of an elongated somatic protrusion of the body.

Ciliates which leave the resting cyst envelopes are on average one half the size of normal trophic cells (Fig. 1, Table 1). They have a complete proboscis and a tail, but the length of the former constitutes only one third of the total length of the whole body, whereas in trophic cells the proboscis makes up almost half of the body length.

Following excystment, the body proportions are adjusted through more intense growth of the proboscis as compared with the rest of the body. Small organisms were found during the first 12 h after excystment. Full adjustment of the proportions requires an additional 24 h.

Growth of excysted cells was examined with regard to 3 features: the elongation of the proboscis, the enlargement of the cytostomal field and the elongation of the somatic portion of the body.

There is no change in the structure of the oral ciliature in the growing proboscis of excysted cells in comparison with the proboscis of normal, trophic cells (Figs. 2,

3, 11). *Dileptus* has a highly specialized oral ciliature (Grain & Golińska, 1969; Kink, 1973; Golińska, 1970). The ventral band of the proboscis is bordered by right and left paracytostomal kineties. Each paracytostomal kinety is composed of pairs of kinetosomes, one non-ciliated and bearing a transverse fibre, the other bearing a cilium and postciliary fibres. The non-ciliated kinetosomes are the closest to the ventral band; the transverse fibres leaving both of these kineties join into one common central fibre running toward the cytostome and dividing the ventral

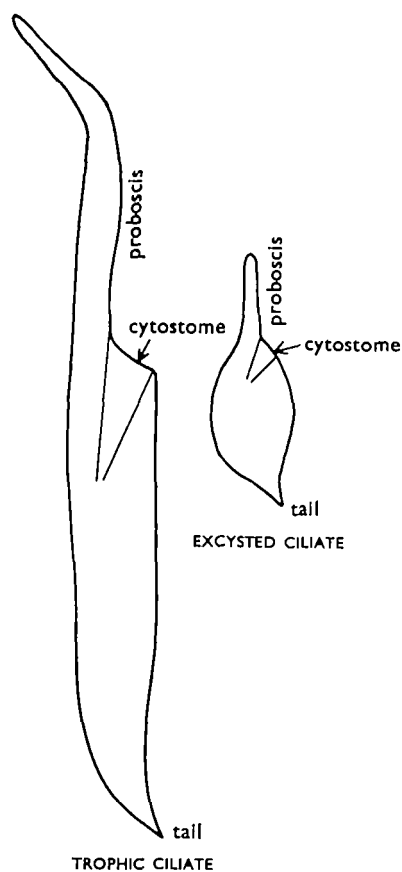


Fig. 1. Comparison of size of trophic and excysted ciliates. The figure shows the outlines of ciliates after staining with protargol.

Table 1. Length of body and proboscis in 10 trophic and 10 recently excysted cells

	Total length of ciliate, $\mu\text{m} \pm \text{s.d.}$	Length of proboscis, $\mu\text{m} \pm \text{s.d.}$	Length of body from cytostome to tail, $\mu\text{m} \pm \text{s.d.}$
Trophic cells	475 ± 67.9	204 ± 53.7	270 ± 35.3
Excysted cells	275 ± 32.4	94 ± 16.0	180 ± 19.5



Fig. 2

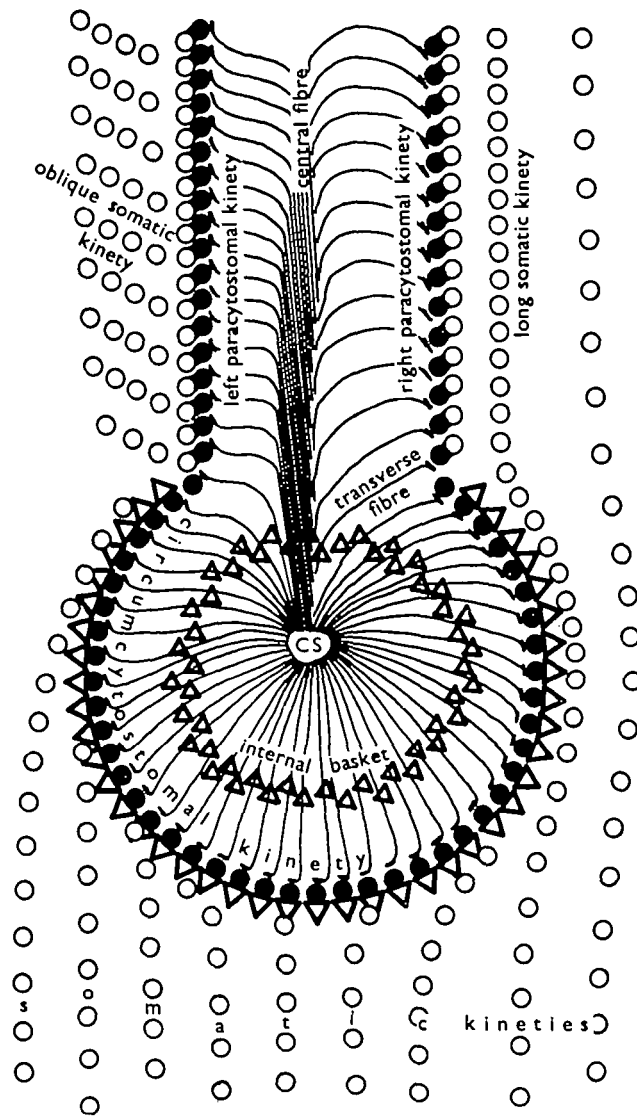


Fig. 3

Fig. 2. *Dileptus* seen from the ventral side. The area bordered by the heavy line corresponds to the area presented in detail in Fig. 3.

Fig. 3. Arrangement of oral and somatic kinetosomes in the trophic ciliate. Black dots indicate non-ciliated kinetosomes, white dots indicate ciliated ones. Lines designate transverse fibres. Large triangles designate nemadesmata of the external basket, small triangles designate nemadesmata of the internal basket.

band of the proboscis into right and left sides. Mucocysts can be observed in the ectoplasm, but only the right side of the band is equipped with toxicysts, which are less numerous in excysted cells than in normal, trophic cells (Figs. 11, 12).

There is no evidence of the formation of new kinetosomal pairs in the territory of the proboscis in excysted cells, in spite of intense growth in this region. Further, kinetosomal pairs do not become more widely spaced along the kinety, since the density of structural elements in the paracytostomal kineties seems to be the same in the normal and the growing proboscis. This leaves us with the possibility that kinetosomal pairs for the elongating paracytostomal kineties differentiate at the base of the ventral band, where the band joins the cytostomal field.

At the base of the ventral band the paracytostomal kineties join the circumcytostomal kinety (Fig. 3). This is composed of single non-ciliated kinetosomes bearing transverse fibres and the nemadesmata of the external, cytopharyngeal basket. In normal trophic cells some of the ciliated kinetosomes of the ventral somatic kineties are situated very close to this circumcytostomal kinety, but do not form kinetosomal pairs.

In excysted growing cells an extensive proliferation of kinetosomes was observed along the circumcytostomal kinety (Fig. 4). This region of kinetosome proliferation in one of the sectioned cells covers a region of about four somatic meridians in width. Some 40 new kinetosomes are formed there simultaneously. It can be observed in the electron micrographs that the kinetosomes are in several different positions in relation to each other, and that they are also in various positions in relation to the cell surface (Figs. 4, 5, 9). These pictures clearly demonstrate that the new oral kinetosomes are in different stages of development. Some of them possess transverse fibres characteristic of mature, oral kinetosomes (Figs. 4, 5).

The sector of the circumcytostomal kinety close to the base of the ventral band is shown in Figs. 4 and 9. Adjacent to the region of intense proliferation, kinetosomes in the state of becoming ordered into pairs are visible – presumably into those pairs which will be incorporated into the elongating paracytostomal kinety of the proboscis. In the right upper corner of the micrographs (Figs. 4, 9) the differentiated pairs are visible. One of the two kinetosomes bears the transverse fibre, the other bears the postciliary fibre, and connexions between kinetosomes are present. Toward the ventral side, the region of proliferation is followed by a region where kinetosomes are ordered perpendicularly to the cell surface (Figs. 4, 9). These kinetosomes are roughly arranged in a double row, but the typical pairs cannot be detected in this region. The region of proliferation presumably supplies kinetosomes for both the elongation of the proboscis and the enlargement of the circumcytostomal kinety. It is not clear whether there exists only one or several sites of proliferation within the circumcytostomal kinety. It is possible that at least two sites of proliferation are present in the growing mouth, with one on each side of the base of the ventral band.

It has already been mentioned above that in the trophic cell each kinetosome of the circumcytostomal kinety bears a nemadesma. These nemadesmata constitute the outer basket of the cytopharyngeal complex (Fig. 6). In growing excysted cells

this basket differs from the basket in the trophic cell. In the latter cells the number of microtubules in a nemadesma varies within the range 50–80 (Kink, 1973), while in excysted cells there are 10–200 microtubules (Figs. 7, 10). The microtubules are arranged in a hexagonal pattern, both in the small and in the large aggregates. In Fig. 8, a section through a nemadesma is shown in which some of the microtubules are arranged in a hexagonal pattern, whereas the rest are not so organized. The thick and thin nemadesmata are disposed randomly and can alternate; sometimes they are scattered along the kinety (Fig. 10). In the region of oral kinetosome proliferation, nemadesmata are situated only on the border close to the cytostome (Figs. 4, 8), so kinetosomal proliferation occurs just outside the existing basket. The distal ends of the nemadesmata, just below their connexion with kinetosomes, are embedded in a microfibrillar material. These pictures of the growing mouth indicate that the external basket of nemadesmata is not fully developed and its elements are still forming while the whole oral apparatus is functioning.

Apart from the external basket, in *Dileptus* there is also an internal basket of nemadesmata enclosed by the external basket (Fig. 4). The internal basket forms a full ring around the cytostome. The distal ends of nemadesmata are embedded in a microfibrillar ring. In the course of stomatogenesis (Golińska & Grain, 1969; Golińska, 1972) this basket is formed in temporary contact with kinetosomes. In excysted cells sections indicating growth of the inner basket of nemadesmata were not found.

The somatic ciliature in *Dileptus* is arranged in longitudinal kineties which terminate posteriorly at the end of the tail; anteriorly the ventral kineties reach the circum-cytostomal kinety, while dorsal kineties terminate at different sites on the narrowing part of the proboscis. Somatic kineties entering the proboscis in close proximity to the ventral band become different from the normal somatic kinety (Fig. 3). The kinety lying next to the right paracytostomal kinety is more densely packed with kinetosomes on the proboscis than in its posterior segment. This kinety is called the long somatic kinety, because it is the longest of all somatic kineties in *Dileptus*. On the left side, the prolongations of somatic kineties appear as numerous, short, oblique rows situated in close proximity to the left paracytostomal kinety. Each oblique kinety is made up of 4 kinetosomes (Kink, 1973). All somatic kinetosomes are equipped with a cilium and a set of fibres including the transverse, postciliary, rootlet fibres and the kinetodesma.

The somatic ciliature in excysted specimens is similar to that in trophic cells (Fig. 4). A basic difference is the density of kineties and kinetosomes on the cell surface; they are more densely packed in excysted cells. It seems that elongation of the somatic part of the body may at least partially depend on growth of the surface between kinetosomes along the kinety.

Proliferation of somatic kinetosomes in *Dileptus* may occur in any stage of the cell cycle, and probably in any region of the somatic ciliature (Golińska, 1972). A sporadic formation of new kinetosomes can be observed on the whole body surface of excysted ciliates. Significantly, more intense proliferation of kinetosomes was observed in the sectors of somatic kineties which are in close proximity to the

circumcytostomal kinety. The proliferation covers both the anterior region of kineties ending at the circumcytostomal kinety (Figs. 5 A, B), and also the sector of the first right kinety, which continues on the proboscis in parallel to the right paracytostomal kinety (Figs. 9, 10). The location of proliferation regions for both somatic and oral ciliature in such close proximity suggests that it is really one region, common for kinetosomes of both kinds.

Within the region of proliferation each category of kinetosomes is formed in its own characteristic way. Oral kinetosomes form a kind of anarchic field when proliferating, where even a fully grown kinetosome may be found lying obliquely to the cell surface, and its transverse fibres may form before the kinetosome is oriented perpendicularly to the surface. A new somatic kinetosome is always formed in direct proximity to an old kinetosome, close to its proximal end and in the region between the kinetodesma and transverse fibre – that is, in a strictly limited area. The young kinetosome at an early stage is shorter and smaller than a mature one, and is located perpendicularly to the proximal end of the mature kinetosome. A position parallel to the mature kinetosome is, however, always acquired before the somatic kinetosome is fully grown, and before its own fibres develop. Therefore it is always easy to distinguish somatic and oral kinetosomes in excysting *Dileptus*, even when they are situated in close proximity.

DISCUSSION

The results obtained reveal some new facts concerning growth processes in excysted cells of *Dileptus*. There is proliferation of oral and somatic kinetosomes during growth. This proliferation was observed in the region of the circumcytostomal kinety and in segments of somatic kineties closest to this kinety. There is no formation of oral kinetosomes on the proboscis, and very feeble proliferation of somatic kinetosomes was observed over somatic regions far from the circumcytostomal kinety.

These facts indicate first that stomatogenesis in *Dileptus* does not terminate with functional maturation of the mouth; second, that there exists a region of basal body proliferation – common to both oral and somatic kinetosomes – which presumably indicates the region where the cell grows.

Growth of mouthparts in relation to stomatogenesis

In ciliates studied so far the process of stomatogenesis consists of several well defined stages (review in Tartar, 1967; Hanson, 1967). There is first the stage of so-called anarchic field formation, in which oral kinetosomes form in a great number. The second stage covers the processes of arrangement of kinetosomes into complicated oral structures. A multiplication of elements of the mouth proceeds during these 2 stages. The last stage comprises the formation of additional mouthparts structure – namely different kind of fibres for the gullet and so on. In these ciliates, if proliferation of oral kinetosomes of well developed mouthparts is still possible, it is very feeble. Mouthparts start to function when all structures are differentiated

(Frankel, 1973; Buhse, Stamler & Corliss, 1973), and it is generally assumed that no growth is possible after the end of differentiation.

The process of stomatogenesis during division and regeneration of *Dileptus*, until the moment when the mouth is functionally mature, has been described previously (Golińska & Grain, 1969; Golińska, 1972). Stomatogenesis in *Dileptus* lacks a well defined anarchic field stage. A future cytostomal field appears and the oral ciliature forms on a line bordering this area. Proliferation begins on the dorsal side and moves towards the ventral side, until the oral ciliature forms a full circle around the cytostomal field. Proliferation is visible in a very short segment and is immediately followed by differentiation of the oral ciliature. Both paracytostomal kineties are thus gradually formed on the right and left sides of the naked cytostomal field and then the circumcytostomal kinety is formed. When the oral ciliature forms a complete ring, nemadesmal baskets are subsequently developed in contact with the kinetosomes of the circumcytostomal kinety.

The growth of the mouth in excysted cells consists of enlargement of the cytostomal field and elongation of oral parts on the proboscis. Proliferation of oral kinetosomes proceeds within the circumcytostomal kinety. Toward the ventral band of the proboscis new kinetosomes are differentiated into pairs and join the paracytostomal kineties. On the ventral side new kinetosomes differentiate into new segments of the circumcytostomal kinety. Thus paracytostomal and circumcytostomal kineties elongate simultaneously.

The results presented indicate that growth of oral parts is still possible after the mouth is functionally mature. Moreover, images of proliferation of oral ciliature resemble those of the 'anarchic field' of other ciliates, except that this anarchic field is situated in proximity to a mature and functioning structure. It remains unknown whether the observed growth is a greatly prolonged stomatogenesis, uniquely characterizing excystment, or whether the mouthparts of *Dileptus* are able to grow in between stomatogeneses during the normal cell cycle. We are trying to answer this question by further study.

The region of proliferation

The results presented reveal a specific pattern of proliferation on the surface of the excysted cell. There is a region close to the cytostomal area, where the whole proliferation of oral kinetosomes takes place and where proliferation of somatic kinetosomes is exceptionally intense. Some characteristics of this region may be defined here: (1) each type of kinetosome proliferates in its own way within this region; (2) the region supplies new kinetosomes for surface growth in several directions; (3) the location of this region on the cell is connected with the location of the cytostome and may be considered as the apical position. Somatic and oral kinetosomes are located very close to one another within the region, yet each type of kinetosome proliferates in its own specific way. Thus each kinetosome is destined from the outset to become either oral or somatic.

The mode of proliferation of oral kinetosomes in excysting *Dileptus* is characteristic

of oral kinetosome proliferation in many other ciliates. It resembles that observed in anarchic fields during stomatogenesis in *Oxytricha* (Grimes, 1973), *Tetrahymena* (Williams & Frankel, 1973), and *Stentor* (Paulin & Bussey, 1971). The mode of proliferation of somatic kinetosomes in *Dileptus* is also characteristic of the formation of kinetosomes in somatic kineties of many other ciliates (Dippell, 1968; Allen, 1969; Bohatier, 1972). What is unusual in the region of proliferation in *Dileptus* is the synchrony of oral and somatic kinetosome proliferation, and the existence of a common region for both.

Other ciliates may possess a region of especially intense proliferation. For instance, during division in *Paramecium* proliferation is restricted to the equatorial part of the cell (Gillies & Hanson, 1968), while in *Spirostomum* the growth of the posterior part is the most intense (Eberhardt, 1962). Proliferation of somatic kineties is not necessarily synchronous with the anarchic field development. In *Tetrahymena* (Nanney, 1971) and *Glaucoma* (Frankel, 1960) a decrease or arrest of somatic proliferation was observed during development of an oral Anlage. Frankel (1960) supposed that developing mouthparts may have an inhibitory effect on proliferation of somatic kineties, at least in kinety 1.

The region of proliferation in *Dileptus* supplies new kinetosomes for elongation of the proboscis, enlargement of the cytopharyngeal armature, and probably for elongation of the whole body. The oral ciliature of the proboscis seems to be formed exclusively at the base of the proboscis. Intense proliferation is also observed within the kinety situated parallel to the right paracytostomal kinety. Kinetosomes are exceptionally dense in this kinety on the proboscis. The intense proliferation in this kinety was observed at the cytostomal level – that is, at the base of the proboscis. The fact that growth of the proboscis is uniform on the right and left sides suggests that the remainder of the somatic kineties on the proboscis may also form their kinetosomes at the base.

The process of enlargement of the circumcytostomal kinety – and hence also enlargement of the external basket of nemadesmata – proceeds by intrusion of new kinetosomes in between the older ones, and by formation of new nemadesmata and transverse fibres. It is not clear whether the inner basket of nemadesmata is also able to enlarge in this way.

In excysted cells of *Dileptus* growth of somatic parts occurs between the cytostome and the posterior end. Proliferation is very extensive within the segments of somatic kineties located near the cytostome, in the proliferation region. Presumably the elongation of the cell body is very intense within or near to the proliferation region. Only sporadic proliferation has been observed in other regions of the somatic kineties.

It seems that the new kinetosomes formed within the proliferation region may be directed toward the posterior pole during body elongation, toward the anterior end of the proboscis during proboscis elongation, or may remain within the proliferation area due to the enlargement of the circumcytostomal kinety.

There is the possibility that the region of proliferation may exist in any trophic cell of *Dileptus*. Some observations indicate that new kinetosomes may form within

the circumcytostomal kinety in any stage of the cell cycle, while proliferation within the paracytostomal kineties was never observed. It is not clear whether growth of *Dileptus* during the normal cell cycle is also most intense near the cytostome, or if this situation is restricted to growth following excystment.

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Fig. 4. Section through the cytostomal field and the adjacent somatic part of the body. In the upper left corner the margin of the cytostomal lip is visible and below it the internal basket (*ib*) of nemadesmata. A continuous line surrounds the region of proliferation of oral kinetosomes adjacent to the circumcytostomal kinety (*cck*); *lsk*, long somatic kinety; *sk*, somatic kinety. Light arrows indicate a pair of oral kinetosomes at the base of the ventral band. Heavy arrowhead indicates new oral kinetosomes with transverse fibre. $\times 11000$.

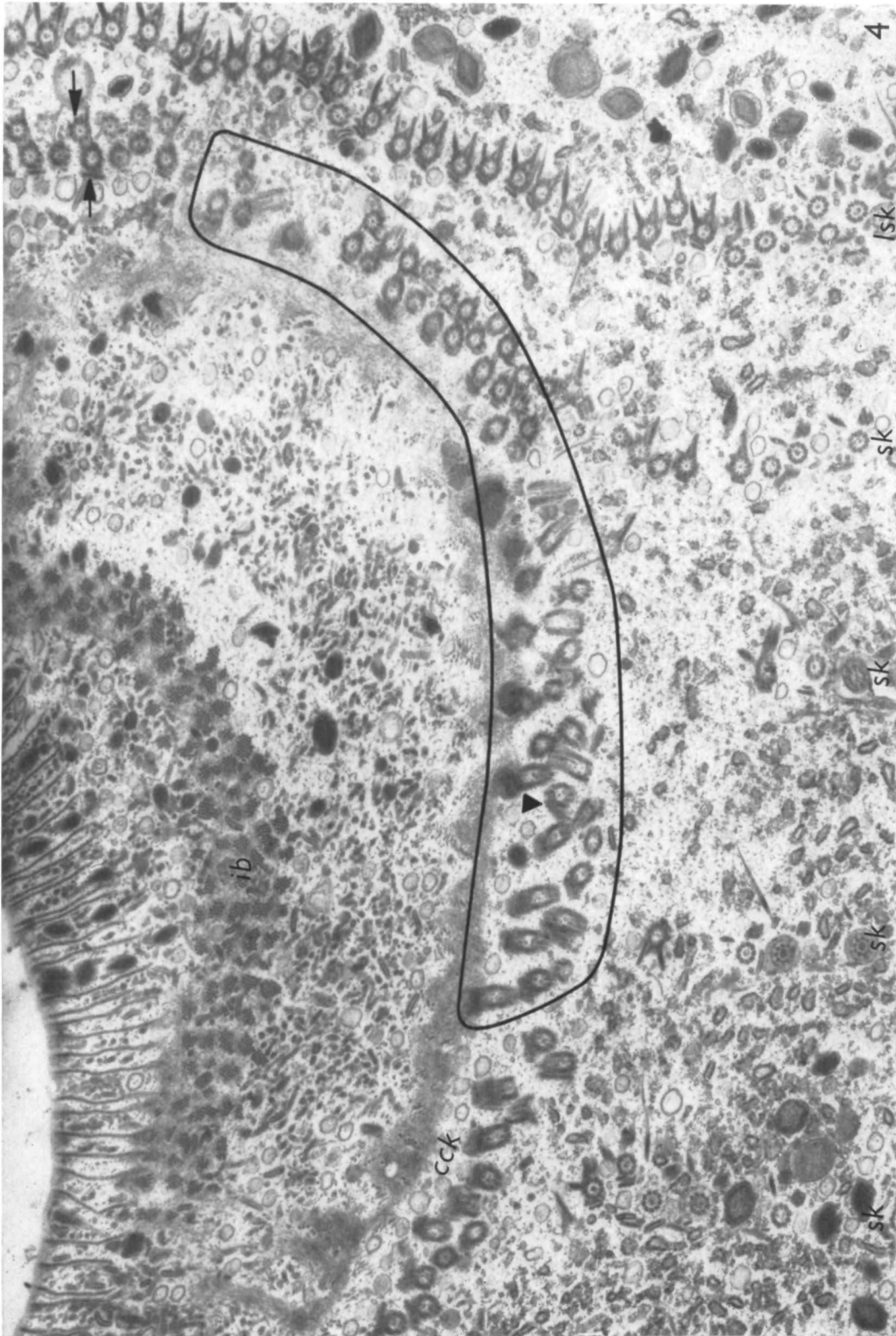


Fig. 5A. Section through the region of proliferation. A section next to the one shown in Fig. 4, nearer to the cell surface. Light arrows indicate the region of proliferation. Heavy arrowheads indicate new oral kinetosomes with transverse fibres. *sk*, somatic kinety. $\times 11000$.

Fig. 5B. Detail of Fig. 5A. *x* indicates new oral kinetosomes. Arrows indicate new somatic kinetosomes. *kd*, kinetodesma; *pc*, postciliary fibre; *t*, transverse fibre. $\times 30000$.

Fig. 6. Disposition of nemadesmata (arrows) in the external basket of trophic ciliate. $\times 30000$.

Fig. 7. Section through the region of oral kinetosome proliferation. Arrows indicate nemadesmata with hexagonal arrangement of microtubules surrounded by microfibrillar material. $\times 15000$.

Fig. 8. Another section through the region of oral kinetosome proliferation. Arrows indicate nemadesmata with microtubules not yet arranged into a hexagonal pattern. $\times 15000$.

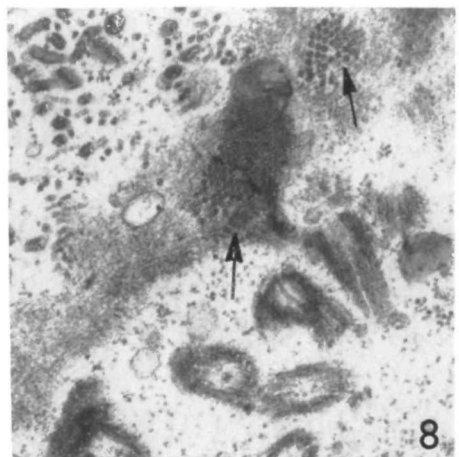
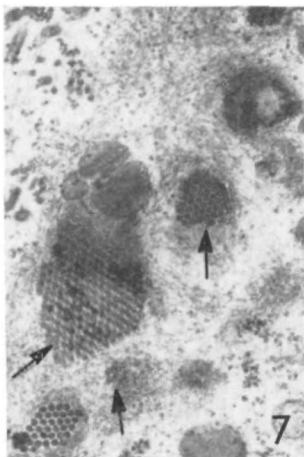
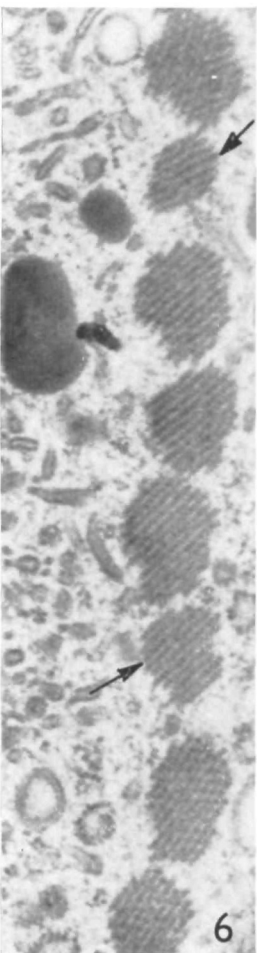
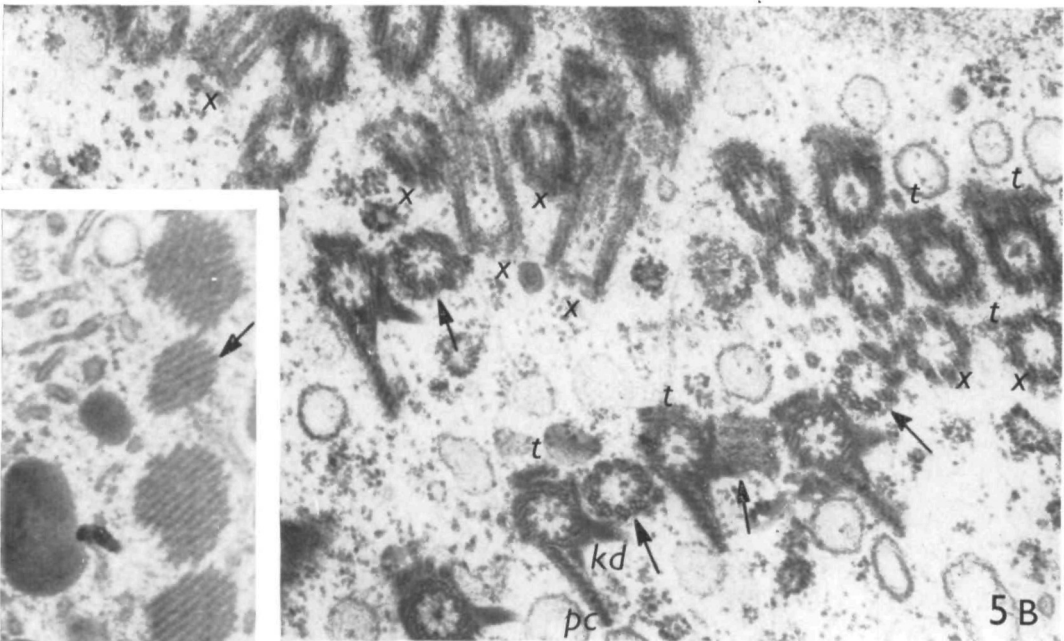
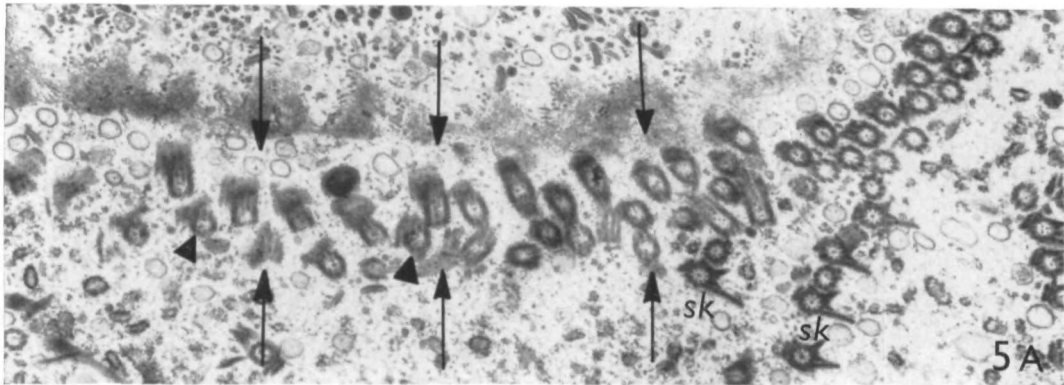


Fig. 9. Section through the region of proliferation close to the base of the ventral band of the proboscis. A discontinuous line surrounds oral kinetosomes becoming ordered into the circumcytostomal kinety. Arrows indicate ordering into the paracytostomal kinety. *lsk*, long somatic kinety; *nok*, new oral kinetosome; *x*, new somatic kinetosomes. $\times 13\ 000$.

Fig. 10A. Section next to that shown in Fig. 9, deeper in the cell. Arrows indicate anterior ends of nemadesmata. *nok*, new oral kinetosome; *x*, new somatic kinetosomes in a long somatic kinety. $\times 12\ 500$.

Fig. 10B. Detail of Fig. 9. Formation of new somatic kinetosoma in a long somatic kinety (arrow). *kd*, kinetodesma; *pc*, postciliary fibre; *t*, transverse fibre. $\times 26\ 000$.

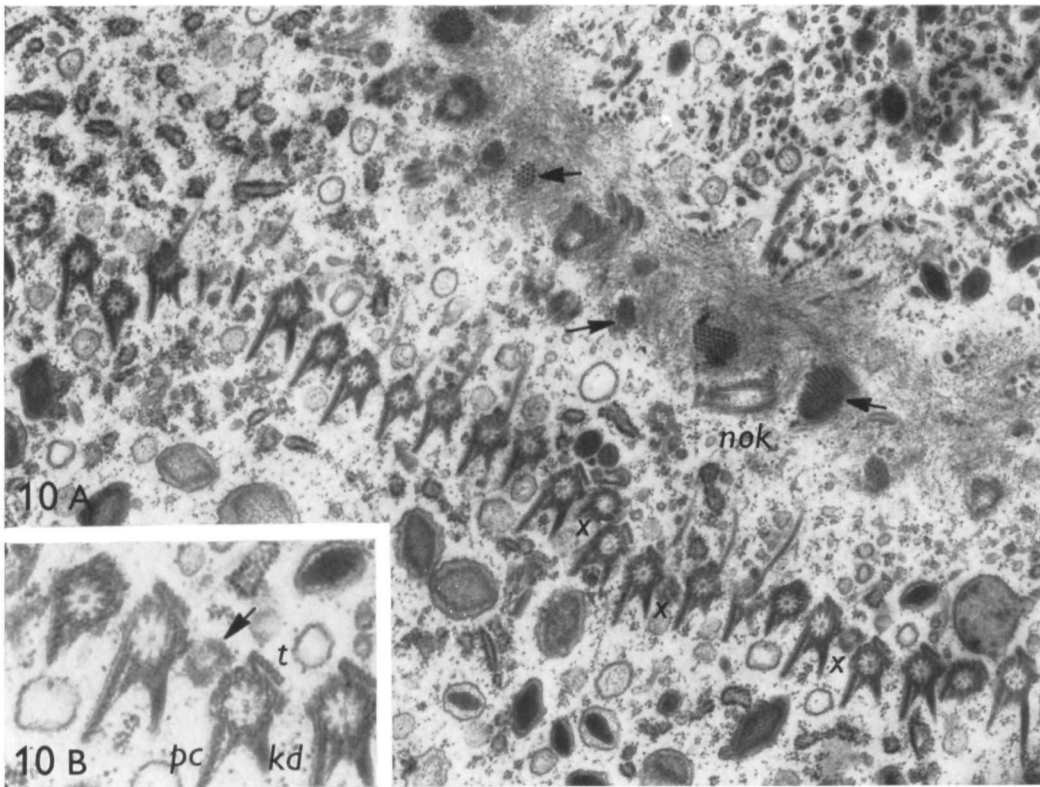
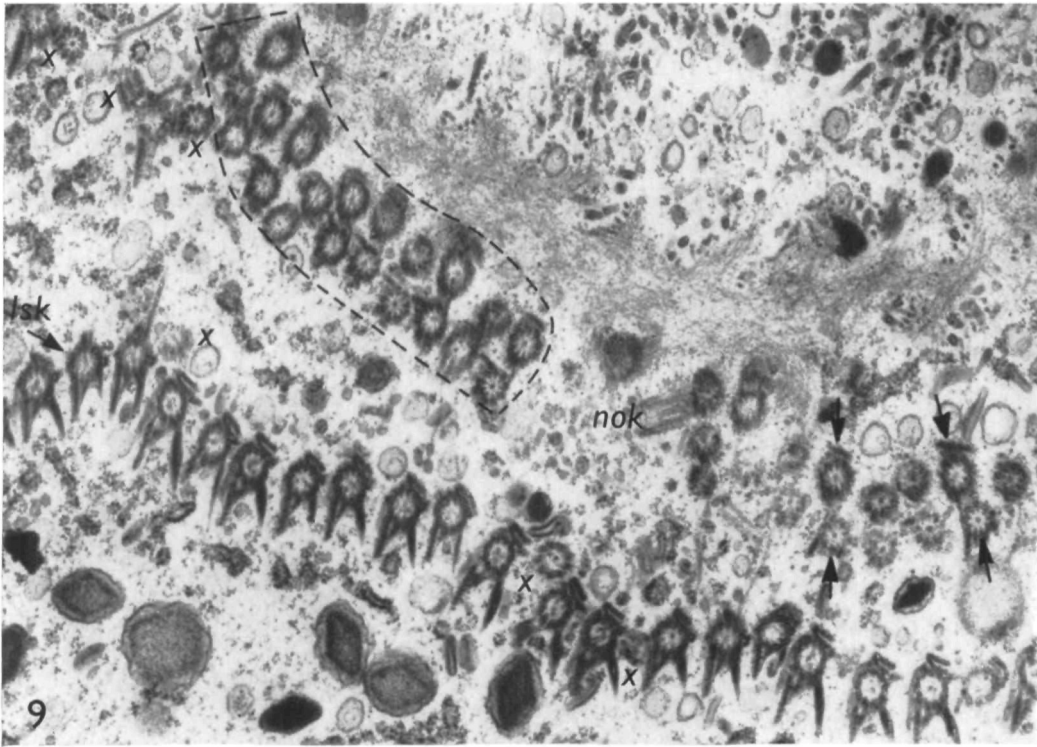


Fig. 11. The ventral band of the proboscis in an excysted ciliate. Toxicysts (*to*) are less numerous than in Fig. 12. *cf*, central fibre; *lpk*, left paracytostomal kinety; *rpk*, right paracytostomal kinety. $\times 9000$.

Fig. 12. The ventral band of the proboscis in a trophic ciliate. Toxicysts (*to*) are numerous in comparison to the excysted ciliate (Fig. 11). $\times 12000$.

