# ORIGIN AND DEVELOPMENT OF FREE KINETOSOMES IN THE FLAGELLATES *DELTOTRICHONYMPHA* AND *KORUGA*

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## SUMMARY

The formation of more than half a million free (non-flagellated) kinetosomes in post-mitotic *Deltotrichonympha* and *Koruga* from *Mastotermes* is described. Ladder-like configurations of prokinetosomes extend from the 2 fibrous walls of the centriolar apparatus in the rostrum. The prokinetosomes within the ladders are arranged side by side in 2 layers. Because the fibrous walls are oriented perpendicular to each other, the 2 groups of prokinetosomal ladders are also mutually perpendicular. The prokinetosomes arise continously next to the fibrous walls, and migrate outward along the ladders as they develop. Consequently, progressive stages in kinetosome formation occur sequentially along the ladders in a polarized fashion. A cartwheel-structure appears first. This is followed by the formation of A tubules, B tubules and C tubules in an orderly sequence around the cartwheel (counter-clockwise, viewed from the distal end). The cartwheel ring disappears after the triplets have formed. The new free kinetosomes accumulate in a disorganized mass at the ends of the ladders. Later, the kinetosomes become organized end to end into the polarized chains found in interphase cells. The fibrous wall of the centriolar apparatus is thus a new type of intermediate structure associated with the mass production of basal bodies. It appears to determine the spatial organization of the 'assembly lines' of developing kinetosomes.

## INTRODUCTION

Although the structure of centrioles and kinetosomes (basal bodies) is well known, many aspects of their origin and development remain enigmatic. Numerous electron-microscope studies have disproved earlier ideas that centrioles reproduce by division (Lwoff, 1950), or by direct template function of pre-existing centrioles. Instead, all centrioles and kinetosomes begin as procentrioles (or prokinetosomes) which develop by the sequential assembly of their component parts (Fulton, 1971; Pitelka, 1974; Wolfe, 1972).

Considerable diversity, however, is shown in the mode of origin of procentrioles. In cells producing a small number of centrioles, the procentrioles typically arise at right angles to existing centrioles (Allen, 1969; Gall, 1961; Dippell, 1968; Robbins, Jentzsch & Micali, 1968). When a large number of basal bodies is formed simultaneously, morphogenesis proceeds through dense intermediate structures that are not themselves centrioles (Anderson & Brenner, 1971; Dirksen & Crocker, 1966;

Dirksen, 1971; Hepler, 1976; Kalnins & Porter, 1969; Mizukami & Gall, 1966; Sorokin, 1968; Steinman, 1968). These intermediate forms may originate in association with centrioles, or they may arise *de novo* in the absence of pre-existing centriolar structures (Hepler, 1976). Morphological discontinuity of basal bodies is a well established feature of the life cycle of many organisms (Cavalier-Smith, 1974; Fulton, 1971; Fulton & Dingle, 1971; Grimes, 1973*a*; Perkins, 1970; Pitelka, 1974). Finally, procentrioles can arise by different pathways even within the same organism (Anderson & Brenner, 1971; Sorokin, 1968; Grimes, 1973*b*).

In this report we describe a unique pattern of mass production of basal bodies. This work is based on our previous discovery of a remarkable accumulation of more than half a million free (non-flagellated) kinetosomes in flagellate protozoa from an Australian termite (Tamm, 1972). Our findings demonstrate a new kind of intermediate structure associated with the origin of prokinetosomes, and reveal several unusual features of kinetosome morphogenesis not encountered elsewhere.

## MATERIALS AND METHODS

*Deltotrichonympha operculata* and *Koruga bonita*, 2 closely related hypermastigote flagellates from the hindgut of the Australian termite, *Mastotermes darwiniensis*, were used in this study. Termites were collected near Darwin, Northern Territory, by Mr R. E. Fox, and shipped by air to the Australian National University, Canberra, where the initial work was done.

In order readily to obtain suitable material (i.e., post-mitotic stages) for studying the formation of free kinetosomes, we devised a new procedure for inducing division of the flagellates (Tamm, in preparation). Briefly, the method consisted of setting up small cultures of *Mastotermes* with known numbers of intermoult and premoult termites. The mitotic index and number of flagellates in certain individuals were followed with time. In contrast to Andrew & Light (1929), we found that an increased incidence of mitosis did *not* occur during refaunation after moulting. Instead, division stages were greatly enriched in intermoult termites which had donated proctodeal food to recently moulted recipients.

Using this method, hindguts from intermoult donors were fixed for electron microscopy by several techniques, as described previously (Tamm, 1972; Tamm & Tamm, 1973*a*, 1976). Flagellates were flat embedded in Araldite to select for appropriate stages. Post-mitotic cells, identified by their small size, triangular shape, and anterior nucleus, were cut out of the Araldite disk and mounted individually on stubs for sectioning in known orientations. Thin sections were cut with a diamond knife, stained with uranyl and lead salts, and viewed in a Philips 300 electron microscope at 80 kV.

## RESULTS

*Interphase cells*

The general features of *Deltotrichonympha* and *Koruga* at the light-microscope level have been reported by Cleveland (1966*a–f*). Using electron microscopy, we recently described the remarkable array of free kinetosomes (Tamm, 1972), and the structure and function of the large centriolar apparatus in these flagellates (Tamm & Tamm, 1973*a, b*).

*Deltotrichonympha* and *Koruga* appear almost identical, ultrastructurally as well as cytologically. Consequently, this study applies to both flagellates. The cells are 300–400 μm long, and conical in shape. Most of the body surface is covered with long
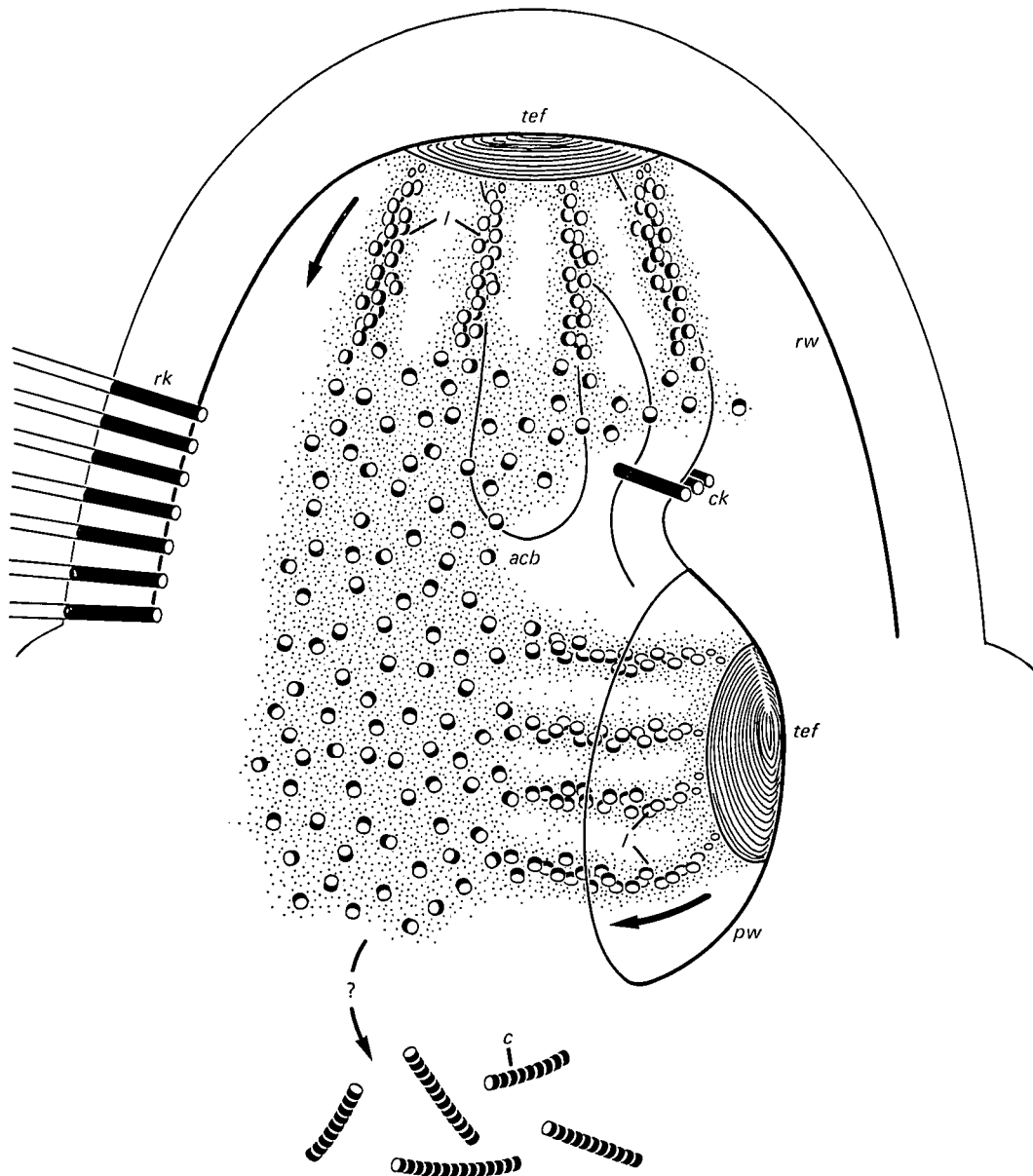
Fig. 1. Diagram of the pattern of short free kinetosome formation in the rostrum of post-mitotic *Deltotrichonympha* and *Koruga*. Kinetosomes are represented as cylinders of different lengths. The centriolar apparatus at this stage consists of the parental part (rostral wall, *rw*; anterior centriolar body, *acb*), and a new posterior part (posterior wall, *pw*) developing perpendicular to the anterior part, but without a second centriolar body yet. The 2 parts are connected by branches which meet at 3 barren centriolar kinetosomes (*ck*). Developing prokinetosomes are arranged side by side in double ladders (*l*) which extend at right angles from the thin elliptical fibre bases (*tef*) of the rostral and posterior walls. Because the 2 walls are oriented perpendicular to each other, the 2 groups of prokinetosomal ladders are also mutually perpendicular. Prokinetosomes develop sequentially along the ladders, beginning with early cartwheel stages next to the fibrous walls (disks), and progressively later stages in an outward direction away from the walls (arrows; gradually thicker cylinders). The new short free kinetosomes accumulate in a central, disorganized mass at the ends of the ladders. Dense granular material (stippled) surrounds the developing kinetosomes. At a later stage, not yet examined, the kinetosomes somehow become organized end to end into the polarized chains (*c*) found between the centriolar apparatus and nucleus in interphase cells (? arrow at bottom). *rk*, rostral flagella kinetosomes, not shown completely. Anterior of cell faces top of diagram.
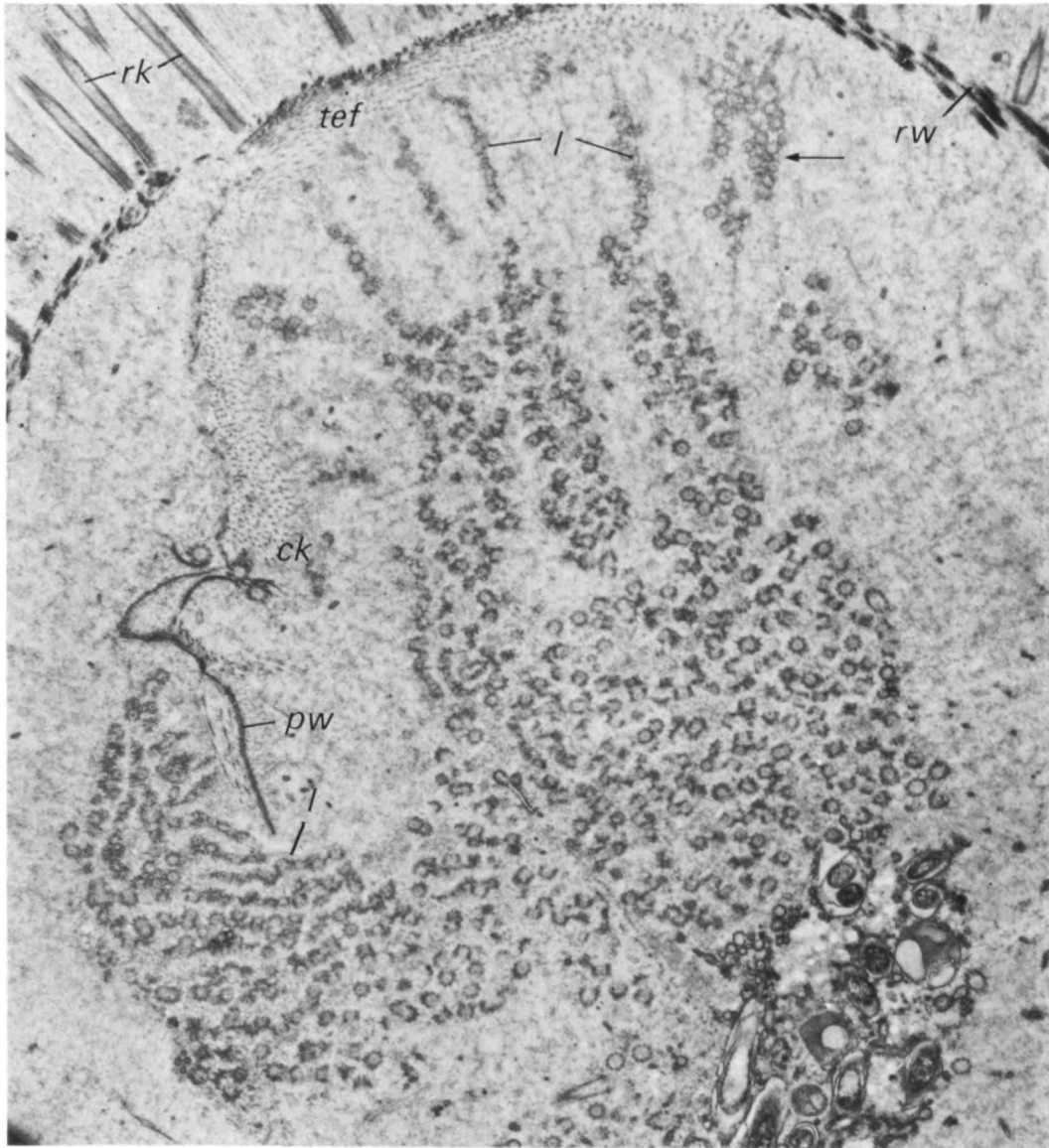
Fig. 2. Survey of the 2 sites of free kinetosome formation in the rostrum. Ladders of developing prokinetosomes (*l*) extend posteriorly from the thin elliptical fibres (*tef*) of the rostral wall (*rw*). and project laterally from the same fibres (not shown) of the posterior wall (*pw*). Face view of prokinetosomes arranged side by side in ladders is shown at upper right (arrow). The nearly completed free kinetosomes are released at the ends of the ladders and accumulate in a jumbled mass in the centre of the rostrum. Dense granular material surrounds the developing kinetosomes. The anterior centriolar body is not evident in this section. *ck*, 3 centriolar kinetosomes; *rk*, rostral flagella kinetosomes. × 14000.

flagella. At the pointed anterior end of the cell is a flagellated cap, the rostrum. Within the rostrum lies the large, complex centriolar apparatus.

## Structure of the centriolar apparatus

The centriolar apparatus is structurally unlike typical centrioles (Tamm & Tamm, 1973 a). It is duplex, consisting of 2 similar parts oriented at right angles to one another (Fig. 1). Each part contains a long, club-shaped centriolar body which projects from the inner side of a concave fibrous wall, The anterior body extends posteriorly from the top of the rostral wall; immediately below and perpendicular to it, the posterior centriolar body projects laterally from a similar fibrous wall lying free inside the rostrum. The 2 parts of the centriolar apparatus are connected by fibrous branches which meet at 3 barren centriolar kinetosomes (Fig. 2).

The walls surrounding the centriolar bodies are made of 4 layers of fibres which differ in structure and orientation (Tamm & Tamm, 1973 a). The innermost layer consists of thin elliptically running fibres; these fibres form the base of the centriolar body and also continue through its length (Figs. 1–5). The centriolar bodies themselves consist of fibrillar and granular material, without recognizable internal symmetry or microtubules.

## Arrangement and structure of free kinetosomes

A remarkable accumulation of more than half a million free (non-flagellated) kinetosomes is found in the anterior cytoplasm of interphase cells (Tamm, 1972). Two classes of free kinetosomes can be distinguished. Five hundred thousand to 750000 short, free kinetosomes, 0·07–0·13 μm long, are concentrated in a dense column which extends from the centriolar apparatus to the nucleus – a distance of ∼70 μm. These kinetosomes are arranged end to end into chains of varying lengths. All of the kinetosomes within a chain face in the same direction with respect to their cartwheel ends.

Seventy thousand to 120000 long free kinetosomes, 0·4–0·7 μm in length, are scattered singly throughout the cytoplasm between the column of short free kinetosomes, and the cell surface. The central axes of the long free kinetosomes lie parallel to the antero-posterior axis of the cell. These kinetosomes are similar in length to the kinetosomes of the body flagella.

Both types of free kinetosomes have a typical kinetosomal ultrastructure (Fig. 6 I–K). Nine triplet microtubules are arranged in a cylinder, ∼0·17 μm in diameter, with a short hub-and-spokes cartwheel at the proximal end. The length of the cartwheel is 0·03–0·05 μm in both types of free kinetosomes.

The pitch of the triplets (defined as the angle between the axis of the triplet row and a tangent to the circumference of the cylinder) is greater at the proximal end of the free kinetosomes, as reported previously for centrioles and flagellated kinetosomes (cf. Fulton, 1971; Pitelka, 1974; Stubblefield & Brinkley, 1967; Wolfe, 1972). In short free kinetosomes, the triplet angle is 40°–50° at the cartwheel end, and 20°–30° at the distal end (Fig. 6 I–K). The outer diameter of the kinetosomes remains fairly constant from one end to the other, but the lumen diameter appears smaller at the
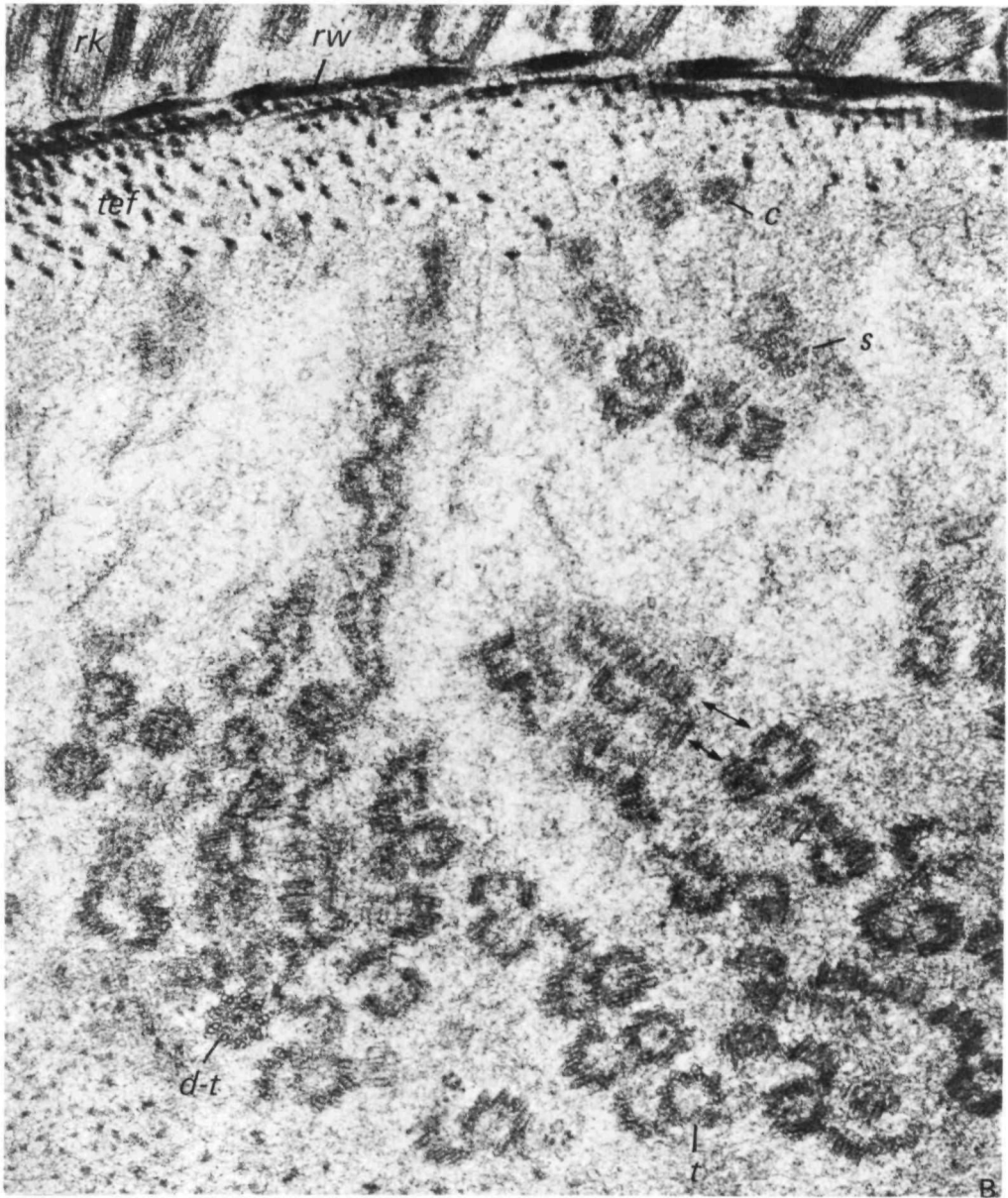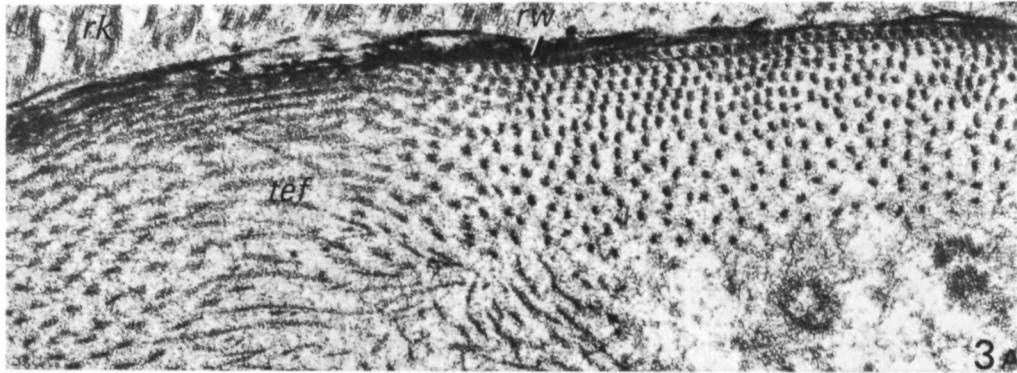
cartwheel end. The decrease in triplet angle from base to apex thus seems to be due to a twisting of the triplet blade away from the centre of the lumen about the C tubule. A similar basis for change in triplet angle along the length of Chinese hamster centrioles was reported by Stubblefield & Brinkley (1967). Measurements of monkey oviduct basal bodies, however, indicated that the triplets are hinged along the A tubule, and rotate inward from proximal to distal ends of the organelle (Anderson, 1972; criticized by Harris, 1973). The reason for these apparent differences in triplet geometry have not yet been resolved.

As in other kinetosomes and centrioles, the free kinetosomes have links between the A and C microtubules of adjacent triplets (Fig. 61, J).

## Cell division

In preparation for division the cells round up, and the nucleus moves anteriorly. The two parts of the centriolar apparatus separate, and each centriolar body becomes a pole of a large, extranuclear mitotic spindle (Tamm & Tamm, 1973 b). The parental rostrum disappears, and a new rostrum for each daughter cell develops in association with each part of the original centriolar apparatus. The fate of the short free kinetosomes, which previously occupied the region now filled with spindle microtubules, has not yet been determined. They are already absent in the earliest division stages we have examined so far. Further studies using earlier stages in mitosis should reveal whether the free kinetosomes ultimately function as basal bodies for flagella.

## Origin and development of free kinetosomes

The formation of the enormous number of short free kinetosomes begins after cell division is completed. At this stage, remnants of the mitotic spindle are often evident by electron microscopy (Fig. 4B). Duplication of the centriolar apparatus takes place by the development of a new posterior part in each daughter cell. In the post-mitotic cells we have examined, most of the fibrous elements of the posterior wall are formed, and the 3 centriolar kinetosomes are present, but the second centriolar body is not yet developed (Figs. 1, 2, 5A).

The short free kinetosomes have a dual origin which reflects the bipartite nature of the centriolar apparatus. Ladder-like configurations of prokinetosomes extend at right angles from the thin elliptical fibre bases of both the rostral and posterior walls (Figs. 1–3, 5). The 2 groups of prokinetosomal ladders are oriented at right angles to each other, corresponding to the mutually perpendicular relation between the 2

Fig. 3. A. Thin elliptical fibre base (*tef*) of the rostral wall (*rw*) where one group of prokinetosomes originates. *rk*, rostral flagella kinetosomes. × 30 000. B. Ladders of prokinetosomes, embedded in dense granular material, extending posteriorly from the thin elliptical fibre base (*tef*) of the rostral wall (*rw*). The prokinetosomes in the ladders are arranged side by side in 2 layers (double arrows). Note progressive stages in prokinetosome development away from the wall (*c*, cartwheel; *s*, singlets; *d-t*, doublets-triplets; *t*, triplets). The cartwheel ring is evident in the lumen of the prokinetosomes. × 44 000.

4A

B
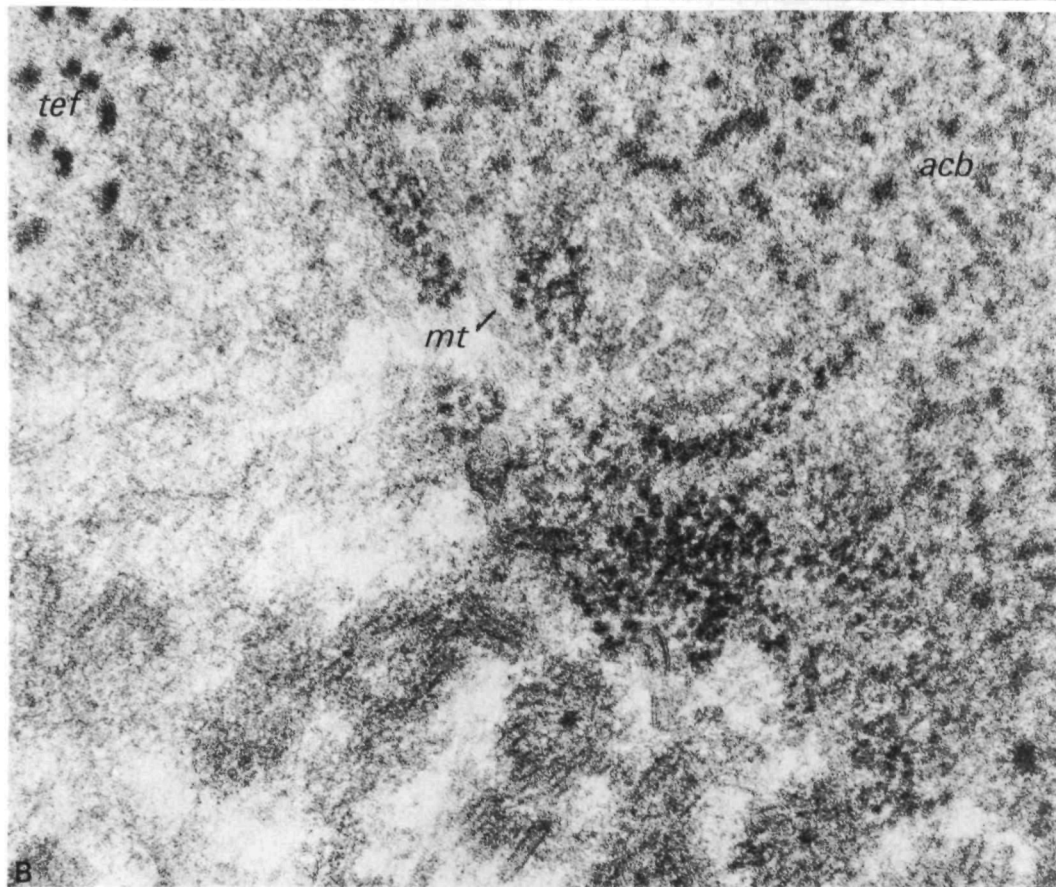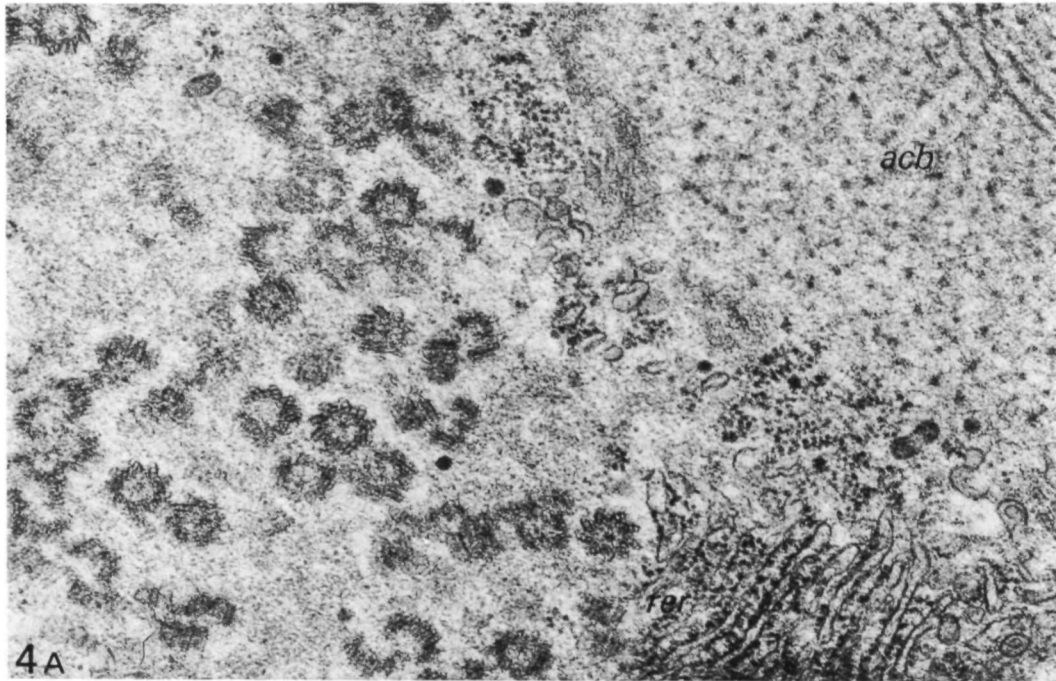
parts of the centriolar apparatus. Within the ladders the developing kinetosomes are arranged side by side in 2 layers, with opposing kinetosomes displaced relative to one another (Figs. 2, 3B, 5). The cartwheel ends of the prokinetosomes in the 2 layers face towards the outside of the ladders (Fig. 5B).

The entire array of developing kinetosomes is embedded in dense granular material (Figs. 1–5). Both free and membrane-bound ribosomes are often found in this material (Fig. 4). The free ribosomes are more numerous than those associated with endoplasmic reticulum, and characteristically occur in small groups (Fig. 4).

Kinetosome morphogenesis proceeds sequentially along the ladders in a polarized fashion, starting at the origin of the ladders next to the thin elliptical fibres (Figs. 1, 3, 5). Here are found cartwheel structures, the earliest detectable stage in prokinetosome formation (Fig. 3B). In addition to a central hub and radial spokes, the cartwheels possess a ring of dense material that encircles the lumen (Fig. 6 A–D). This ring is somewhat variable in thickness, and often appears wavy or scalloped in outline. It has an internal diameter of ~60 nm, and a more irregular outside diameter of 70–80 nm.

Progressively later stages in prokinetosome development are encountered along the ladders in an outward direction away from the walls. Single A tubules form first around the outside of the cartwheel ring (Figs. 3B, 5C, 6B–D). The scalloped appearance of the ring is most evident at this stage, and the ridges of the ring coincide with the terminations of the radial spokes (Fig. 6D). The A tubules form outside the ring at intermediate positions between the spoke radii (Fig. 6C–E). As a result, adjacent tubules are always spaced 40° from one another around the ring (Fig. 6D). The new A tubules are thus not in direct contact with the radial spokes (which point midway between them). Only later, when the ring disappears, do the spokes directly attach to the A tubules (see below).

Images suggesting both a sequential (Fig. 6D) and a random (Fig. 6C) order of formation of A tubules around the cartwheel have been obtained. When development appears orderly, the A tubules seem to be initiated in a counter-clockwise direction (viewed from the distal end) around the ring (Fig. 6D; see also B and C tubule formation below). Longitudinal sections through these early stages show that the cartwheel-singlet complex is only 20–50 nm in length.

After the A tubules have formed, B tubules appear on their peripheral walls as outgrowths that curve around and re-attach to the walls more medially (Fig. 6D, E). C tubules develop similarly to complete the triplets (Fig. 6E). The pattern of doublet and triplet formation indicates that the initiation of the B and C tubules proceeds sequentially in a counter-clockwise direction around the wall of the prokinetosome

Fig. 4. Groups of free and membrane-bound ribosomes in the dense granular material surrounding developing prokinetosomes. Free ribosomes are more numerous than those associated with endoplasmic reticulum (*rer*, A); in B, free ribosomes are located near the thin elliptical fibres (*tef*) of the rostral wall; remnants of mitotic spindle microtubules (*mt*) are still associated with the anterior centriolar body (*acb*). A, × 39 000; B, × 83 000.
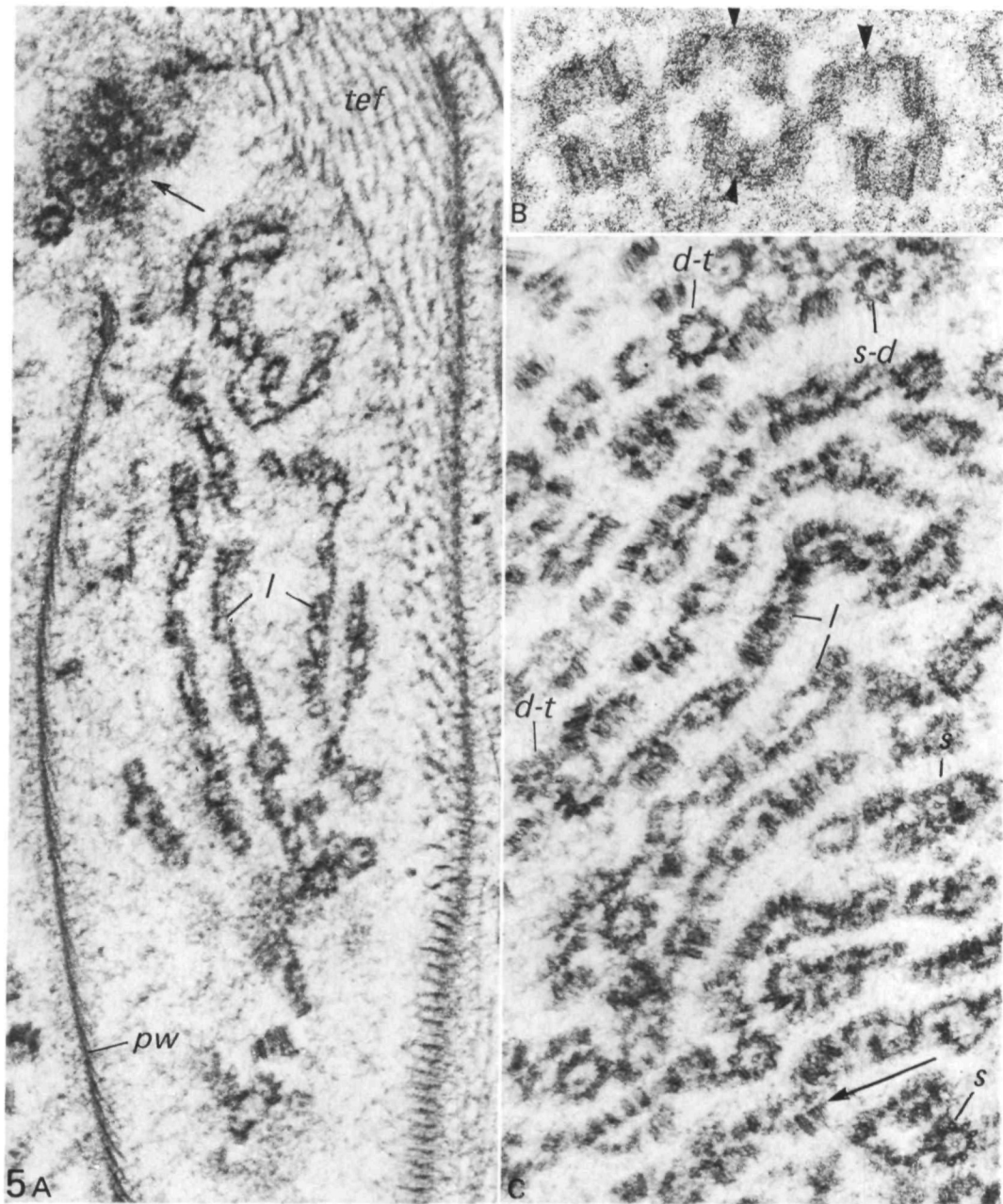
Fig. 5. Structure of prokinetosomal ladders (*l*) at the posterior site of origin. The posterior wall (*pw*) and thin elliptical fibre base (*tef*) are evident in A, but are out of the field of view to the right in C. Tangential views of ladders show clusters of prokinetosomes side by side in cross-section, embedded in dense granular material (arrow, upper left in A). Longitudinal aspects show developing prokinetosomes arranged laterally in 2 layers, with opposing kinetosomes displaced relative to one another (B, C). The cartwheel ends of the prokinetosomes in the 2 layers face toward the outside of the ladder (arrowheads, B). Prokinetosome development proceeds progressively along the ladders in a direction away from the wall, as shown in C (arrow; *s*, singlet stage; *s-d*, singlets-doublets; *d-t*, doublets-triplets). A, ×40000; B, ×80000; C, ×45000.

(viewed from the distal end) (Fig. 6E). Moreover, the process of tubule initiation often begins at a similar radial position in the walls of adjacent prokinetosomes (Fig. 6E).

As in other procentrioles (Dippell, 1968; Pitelka, 1974), the triplet angle in the developing free kinetosomes is steeper than in the mature organelles. The greatest triplet angle (70°) is found at the extreme proximal end, before the appearance of A–C links (Fig. 6F).

During tubule elongation and subsequent maturation, the triplet angle decreases and the lumen diameter increases, without significant change in the outside diameter of the cylinder. Thus, variation in triplet angle during kinetosome development has the same basis as the changes in triplet angle along the length of mature kinetosomes, i.e. the triplets appear hinged along the C tubule.

The cartwheel ring gradually breaks down and disappears (Fig. 6E, G, H). The radial spokes, which earlier did not appear connected to the triplets, now extend the full distance from the central hub to the A tubules. A–C links also become evident at this stage.

At the ends of the ladders away from the walls, the kinetosomes are approximately the length of the short free kinetosomes in interphase cells. These nearly completed free kinetosomes are released from the 'assembly lines', and accumulate in a disorganized array around the centriolar apparatus (Figs. 1, 2). At some later stage, not yet examined, these kinetosomes become organized end to end into the polarized chains found in interphase cells.

DISCUSSION

Previous electron-microscope studies have shown that centrioles and basal bodies arise as procentrioles (or prokinetosomes) which develop the 9-fold triplet structure through a stepwise assembly process (Fulton, 1971; Pitelka, 1974; Wolfe, 1972). In most cases, the hub-and-spokes cartwheel appears first (except in *Paramecium*; Dippell, 1968), followed by the sequential formation of single A tubules equally spaced around the circle (Anderson & Brenner, 1971; Kalnins & Porter, 1969; Perkins, 1970). Additional microtubules are added in an orderly fashion around the wall of the procentriole to form doublets, then triplets (Anderson & Brenner, 1971; Kalnins & Porter, 1969). Elongation of the tubules occurs mainly at the distal end (Anderson & Brenner, 1971; Wolfe, 1972), although growth at the proximal end has also been reported (Grimes, 1973*b*). At both ends, elongation of tubules within the triplets is unequal (Anderson & Brenner, 1971; Grimes, 1973*b*). In many cases the cartwheel disappears shortly after the centriole or basal body is formed (Anderson & Brenner, 1971; Kalnins & Porter, 1969; Wolfe, 1972).

The development of free kinetosomes in *Deltotrichonympha* and *Koruga* follows this same general pattern. The appearance of a cartwheel with 9-fold symmetry before any microtubules are visible supports the view that the cartwheel has a role in organizing the pattern of tubule assembly (Anderson & Brenner, 1971; Kalnins & Porter, 1969; Perkins, 1970; Steinman, 1968; Stubblefield & Brinkley, 1967; Wolfe, 1972). The cartwheel ring is a prominent feature of the prokinetosomes, but gradually
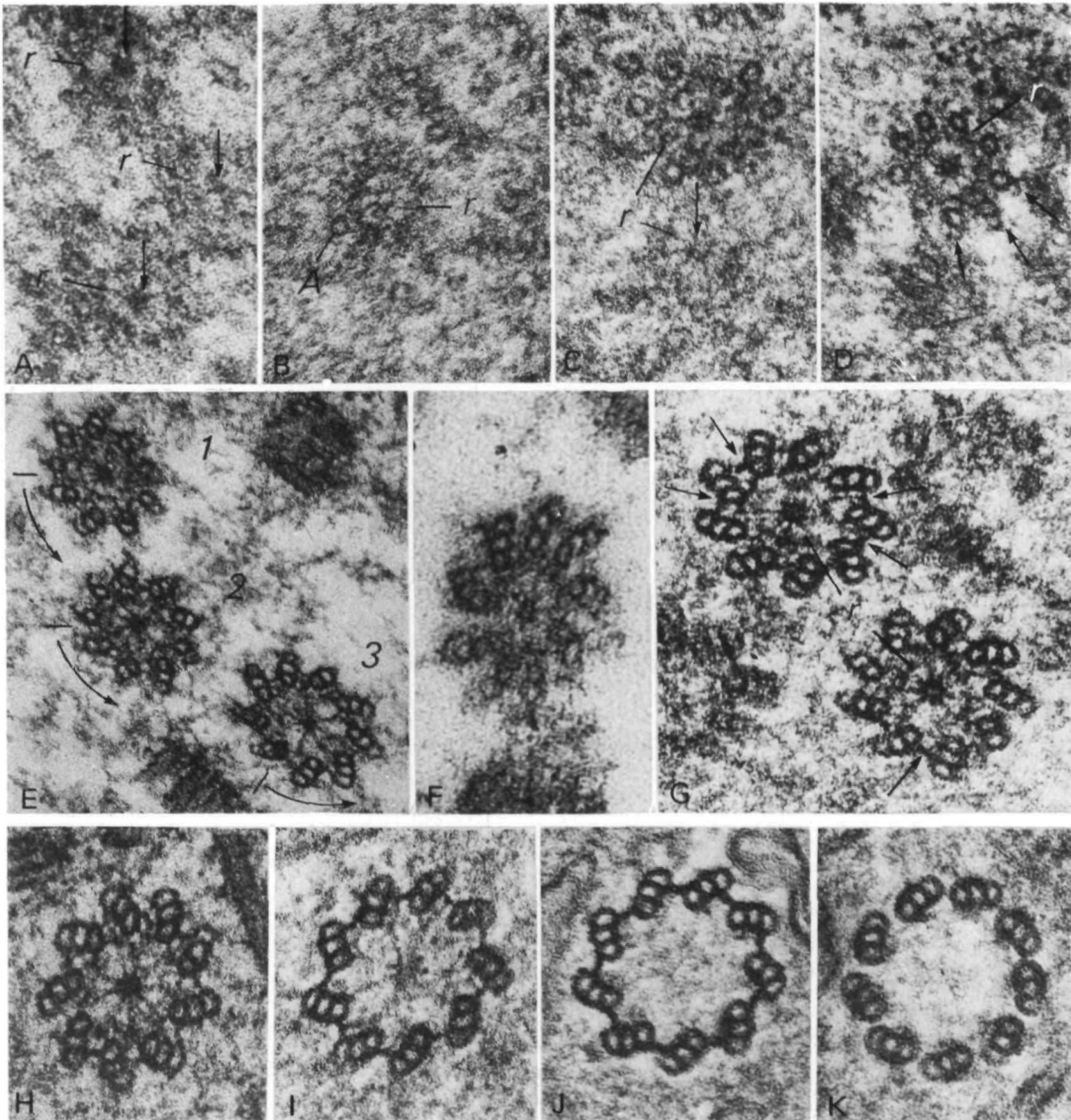
Fig. 6. A–H. Cross-sections of short free kinetosomes in various stages of development, arranged in chronological sequence.

A. 3 cartwheels prior to the appearance of microtubules. Note the dense fuzzy ring (r) surrounding the hub (arrow) and spokes of each cartwheel. × 140000. B. Cartwheel with the first A tubule (A) formed outside the ring (r) × 122000. C. Prokinetosome (upper) with 6 A tubules around the cartwheel ring (r). Three gaps in the sequence of tubules exist at 120° intervals around the ring. Neighbouring A tubules are spaced 40° of arc apart, and are 80° distant across a gap. Note the scalloped appearance of the cartwheel ring. The lower cartwheel represents an earlier stage before A tubules appear (arrow points to central hub). × 140000. D. Prokinetosome with 9 equally spaced A tubules, and several incomplete B tubules on the lower right wall (arrows). The A tubules to the immediate left of the ones with forming B tubules appear fainter, and the A tubule walls become denser in a clockwise direction around the ring. This suggests that the tubules develop in sequential order, counterclockwise

disappears during development. Likewise, the hub-and-spokes is fainter in mature free kinetosomes than in forming ones. This suggests that the ring as well as the cartwheel serves a morphogenetic role in prokinetosome formation.

However, in contrast to other reports (Anderson & Brenner, 1971; Kalnins & Porter, 1969; Perkins, 1970), we found that the new A tubules are not attached to the spokes of the cartwheel. Instead, the tubules arise outside the cartwheel ring at positions intermediate to the spoke radii. If the initial 9-fold symmetry of the cartwheel dictates the placement of the A tubules, as suggested for monkey oviduct basal bodies by Anderson & Brenner (1971), then it does so in a more indirect and less obvious manner in the free kinetosomes.

Observations on the graded pattern of incomplete doublets and triplets around the lumen indicate that the initiation of the B and C tubules proceeds sequentially around the wall of the prokinetosome. Although our evidence for sequential formation of the A tubules is not as clear, it seems likely that the assembly of the singlets also follows a similar pattern. Viewed from the distal (apical) end, tubules are initiated in a counter-clockwise direction around the prokinetosome. In contrast, all other workers report a clockwise sequence of tubule formation around procentrioles (viewed in the same orientation) (Anderson & Brenner, 1971; Dippell, 1968; Kalnins & Porter, 1969). The reason for this apparent difference is not known.

In this regard, it should be noted that tubule formation consists of an initiation and a growth phase (Anderson & Brenner, 1971). Tubule growth is a non-uniform process, and occurs non-sequentially around the procentriole (Anderson & Brenner, 1971).

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around the cartwheel (the angle of the incomplete doublets shows that the prokinetosome is viewed from apex to base) (see also E). Note that the cartwheel ring (*r*) appears scalloped, with the radial spokes pointing to the ridges; the A tubules lie midway between the spoke-ridge radii, and appear not to be directly attached to the spokes at this stage. × 111000. E. Prokinetosomes engaged in the formation of B and C tubules. Tubules are initiated on the outer wall of the previously formed tubule, and curve around clockwise to re-attach to the wall more medially. The 3 prokinetosomes are viewed from the distal (apical) end, as in D. Progressive stages in formation of B and C tubules occur clockwise around the lumen in all 3 prokinetosomes, beginning at a similar radial position in the wall of each (bar). The earliest stage is just above the bar, and the most complete triplet is immediately below the bar (i.e., a partially formed B tubule is adjacent to and above a partially formed C tubule in prokinetosomes *1* and *2*). Therefore, tubule initiation occurs counter-clockwise around the prokinetosomes (curved arrows), as in D. The cartwheel ring is evident in prokinetosomes *1* and *2*, but is partially missing in prokinetosome *3*. Note that the radial spokes point to positions midway between the A tubules, and do not contact the tubules directly (cf. prokinetosome 3). × 98000. F. Steep triplet angle of ~ 70° at the proximal end of a prokinetosome. A–C links have not yet formed. × 128000. G. Prokinetosomes with 9 complete triplets (upper) and an almost complete set (lower). The cartwheel ring (*r*) is breaking down, as shown by gaps in its circumference. A–C links are forming (arrows). × 114000. H. Proximal end of a prokinetosome with the cartwheel ring almost completely gone. A–C links are present between most triplets. × 135000. I–K. Cross-sections through mature short free kinetosomes at different levels. × 135000. I. Proximal cartwheel end. Compared to prokinetosomes, the triplet angle has decreased, the cartwheel is fainter, A–C links are completed, and the spokes now attach directly to the A tubules. J. More distal level. A–C links are still evident. K. Distal end. A–C links are absent and the triplet angle is reduced compared to the proximal end (I).

Differing rates of tubule elongation within the same triplet can produce images which mimic stages in the formation of the triplet pattern (Grimes, 1973*b*). Although we have not serially sectioned prokinetosomes to distinguish clearly between initiation and growth of the tubules, the regularity of the pattern we find suggests that the images probably represent stages in the initiation process.

The dense granular material which surrounds the prokinetosomes is not found with the mature short free kinetosomes of interphase cells. This material presumably represents precursors for kinetosome assembly; it is probably synthesized on the polyribosomes found scattered throughout it.

In contrast to this standard pattern of maturation, the origin of procentrioles shows considerable diversity. In many cells which are doubling their number of centrioles, new centrioles arise perpendicular and adjacent to the proximal end of pre-existing centrioles (Allen, 1969; Gall, 1961; Dippell, 1968; Robbins *et al.* 1968). When a large number of centrioles is formed simultaneously, procentrioles arise perpendicular to the surface of electron-dense aggregates of fibrous or granular material, variously referred to as deuterosomes, condensation forms, procentriolar organizers, or blepharo- plasts (Anderson & Brenner, 1971; Dirksen & Crocker, 1966; Dirksen, 1971; Hepler, 1976; Kalnins & Porter, 1969; Mizukami & Gall, 1966; Sorokin, 1968; Steinman, 1968). In the differentiation of ciliated epithelia, these intermediate structures appear initially in association with pre-existing centrioles, but later become separated from them. In the development of flagellated sperm of lower plants, however, the intermediate structure, or blepharoplast, arises in the absence of pre-existing centrioles (Hepler, 1976). *De novo* formation of basal bodies in cells that previously lacked them is now well documented in other plants and animals (Cavalier-Smith, 1974; Fulton, 1971; Fulton & Dingle, 1971; Grimes, 1973*a*; Perkins, 1970; Pitelka, 1974).

The present study shows that the free kinetosomes of *Deltotrichonympha* and *Koruga* arise in close association with an unusual type of intermediate structure, the centriolar apparatus. The centriolar apparatus of these flagellates can thus be added to the already diverse list of intermediate structures, not themselves centrioles, which are associated with the mass production of procentrioles. Like the blepharoplast of *Marsilea* (Hepler, 1976), the centriolar apparatus first serves as an organizing centre for mitotic spindle microtubules, and then participates in the formation of a large number of basal bodies.

The precise role of the various kinds of intermediate structures in centriole morpho- genesis is unknown. They have been postulated to organize procentrioles, and to provide precursor material for centriole assembly (see Fulton, 1971). The duplex pattern of kinetosome formation in *Deltotrichonympha* and *Koruga* points to a specific contribution of the intermediate structure in this cell. The finding that the orientation of the prokinetosomal ladders is the same with respect to each part of the centriolar apparatus, even though the 2 parts are mutually perpendicular, indicates that the intermediate structure in this case may determine the spatial organization of the newly forming kinetosomes.

The specific component of the centriolar apparatus that plays this role in kinetosome formation can be tentatively identified by a process of elimination. The rostral kineto-

somes cannot be involved, because they are not present at the posterior site of origin. At the stage when prokinetosomes arise, the posterior wall has formed but the posterior centriolar body has not yet appeared, ruling out a possible role of the centriolar body in kinetosome formation. This leaves the fibrous wall as the major structural element present at both sites of origin of prokinetosomes. The wall, particularly the base of thin elliptical fibres, may thus be the intermediate structure which determines the spatial orientation of the prokinetosomal ladders.

The origin of the fibrous wall itself is not understood. However, the 3 centriolar kinetosomes, which are already duplicated before cytokinesis, may play a role in the development of the posterior wall for each daughter cell (Tamm & Tamm, 1973 *b*). If so, the fibrous wall, like many other intermediate structures involved in large-scale production of basal bodies, would originate in association with pre-existing centrioles, and not *de novo*.

The spatial pattern of prokinetosome formation in *Deltotrichonympha* and *Koruga* differs in 2 major respects from other systems of mass production of basal bodies (Anderson & Brenner, 1971; Dirksen & Crocker, 1966; Dirksen, 1971; Hepler, 1976; Kalnins & Porter, 1969; Mizukami & Gall, 1966; Sorokin, 1968; Steinman, 1968). First, in other cells the procentrioles arise at right angles to the surface of the intermediate structures; in *Deltotrichonympha* and *Koruga* the prokinetosomes originate side by side in double ladders which extend outward from the intermediate structure or fibrous wall. Secondly, the development of large numbers of basal bodies is a synchronous process in other systems. During the formation of free kinetosomes in the termite flagellates, however, the prokinetosomes arise continuously next to the thin elliptical fibres of the wall, and migrate outward along the ladders as they develop. This assembly line process is not surprising, since the total number of kinetosomes produced in these cells is many times greater than in other systems.

The new short free kinetosomes are released from the bottoms of the ladders and accumulate in a disorganized mass around the centriolar apparatus. At some later stage these kinetosomes become organized end to end into the polarized chain seen in the column of interphase cells. The mechanism responsible for the migration and marshalling of more than half a million kinetosomes into this highly ordered array is still a complete mystery.

The kinetosomal chains do not represent a stage in kinetosome formation, as originally supposed. Instead, the chains are evidently an accumulation of young kinetosomes, arrested in an early stage of growth, and stored end to end for an unknown reason.

The relationship between the short and long free kinetosomes is still not clear, but several observations suggest that at a certain stage in the cell cycle the short free kinetosomes become long ones by growth at the distal end (Tamm, 1972). The 2 populations of free kinetosomes may thus have a common origin, as set forth in this paper, and simply represent different stages of growth. Future work is aimed at discovering the function of this enormous number of free kinetosomes, as well as the mechanism of their spatial patterning during interphase. In addition, this system should prove useful for investigating the biochemistry of kinetosomes.

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