

FREE KINETOSOMES IN AUSTRALIAN FLAGELLATES

I. Types and Spatial Arrangement

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ABSTRACT

An electron microscope study was made of *Deltotrichonympha* and *Koruga*, two closely-related hypermastigote flagellates that live in the hindgut of the Australian termite, *Mastotermes darwiniensis*. These symbiotic protozoans have a typical flagellated rostrum and long body flagella. Their "giant centrioles" (centriolar apparatus) are large, fibrillar, and granular bodies which do not resemble typical centrioles in structure. The unique feature of interphase cells is the presence of more than half a million free kinetosomes in the anterior cytoplasm. Two classes of free kinetosomes, differing in length and spatial arrangement, were found. 500,000–750,000 short free kinetosomes are concentrated in a dense column which extends from the centriolar apparatus in the rostrum to the anterior side of the nucleus. Most of the short free kinetosomes in the column are arranged end-to-end in chains of varying lengths. Within a kinetosomal chain, all of the individual kinetosomes face in the same direction with respect to their cartwheel ends. In most flagellates, the short free kinetosomes are 0.07–0.13 μ long, and are remarkably similar in length within any cell. Occasionally, cells with uniformly "longer" short free kinetosomes are found. 70,000–120,000 long free kinetosomes are scattered singly throughout the cytoplasm between the column of short free kinetosomes and the cell surface. These long free kinetosomes are 0.4–0.7 μ long, similar in length to the kinetosomes of the body flagella, and are oriented parallel to the anterior-posterior axis of the cell. The significance of this remarkable accumulation of free kinetosomes is discussed.

INTRODUCTION

Ever since the first duckbill platypus arrived in London more than 160 years ago, the wildlife of Australia has been a source of surprise and interest to biologists. This report is the first in a series of papers dealing with the remarkable concentration of free kinetosomes and the unusual centriolar apparatus found in protozoans from the Australian termite, *Mastotermes darwiniensis*.

The symbiotic flagellate protozoans which inhabit the hindgut of various termites and the wood-eating roach, *Cryptocercus*, have been described extensively on the light microscope level (see references 3 and 20 for bibliographies). Taking advantage of sexual reproduction in the flagellates

of *Cryptocercus*, Cleveland has studied the diverse sexual processes and chromosome behavior of these flagellates (4), as well as the life cycles of their giant "centrioles" (5).

Where these older cytological observations have been reinvestigated with modern techniques, fascinating results have emerged. For example, recent electron microscope studies on the giant centrioles of these flagellates have revealed large fibrillar bodies, without a typical centriolar ultrastructure, which nevertheless function as centers for the mitotic spindle at cell division (18, 19).

Sutherland's (26) discovery of short cilia, in

addition to several flagella, on *Mixotricha paradoxa*, a large protozoan from *Mastotermes*, prompted Cleveland and Grimstone (14) to reinvestigate this organism with the electron microscope. They found that the short cilia were really attached spirochaete bacteria, whose coordinated undulations propelled the protozoan. This unusual finding has intrigued workers on ciliary coordination (25), as well as students of the origin of eucaryotic cells (22).

Cleveland's (6-10) light microscope description of *Deltotrichonympha* and *Koruga*, the two other conspicuous genera of flagellates found in the hindgut of *Mastotermes*, has likewise caused us to reexamine these protozoans with the electron microscope. By so doing, we have discovered several new things about kinetosomes and centrioles. In this paper we describe the remarkable accumulation of more than half a million free kinetosomes found in the cytoplasm of *Deltotrichonympha* and *Koruga*. In subsequent papers, we will describe the unique mode of formation of the free kinetosomes¹, and the fine structure and function of the centriolar apparatus of these flagellates².

MATERIALS AND METHODS

Animals

The primitive termite *Mastotermes darwiniensis* was collected in Australia near Townsville, Queensland, and Darwin, Northern Territory. Termites were shipped by air to the Australian National University, Canberra, where they were kept successfully for months in tins of dead wood at 80°F.

The hindgut of *Mastotermes* harbors three conspicuous genera of large, flagellate protozoans: *Koruga*, *Deltotrichonympha*, and *Mixotricha*. The latter two protozoans were first discovered by Sutherland (26). Recently, all three genera have been described on the light microscope level by Cleveland (6-13). Cleveland (9) recognized *Koruga bonita* as a new genus and species of hypermastigote flagellate, closely related to *Deltotrichonympha*. In addition, Cleveland (6) distinguished *Deltotrichonympha operculata* from *Deltotrichonympha nana*, the latter species being half the size of *D. operculata*. This paper deals only with *Deltotrichonympha operculata* and *Koruga bonita*.

The termites used in this study were adult members of the functional worker caste (16).

¹ Tamm, S. L. Manuscript in preparation.

² Tamm, S., and S. L. Tamm. Manuscript in preparation.

Observation of Living Material

To examine living flagellates, a hindgut was removed from a termite, opened in a drop of 0.6% NaCl on a microscope slide, and quickly covered with a Vaseline-ringed cover slip. The flagellates remained motile for 10-15 min or longer under these conditions, whereas exposure to air for a few minutes killed these anaerobic protozoans. Thousands of flagellates were obtained from a single termite. Living flagellates were photographed with Zeiss Nomarski interference-contrast optics, using a Zeiss microflash and a 0.65 NA objective.

Electron Microscopy

Hindguts were opened in a small volume of 0.6% NaCl, to which a large volume of fixative was immediately added. Several fixation methods were tried, with no significant differences observed between them. Because the original purpose of the investigation was to preserve the flagellar and body waves, an instantaneous fixation procedure was used initially. Flagellates were fixed in a 2.6% osmium tetroxide, 2.3% mercuric chloride solution for 15 min at room temperature (modified from Parducz [23]). Other batches of flagellates were fixed in 2% osmium tetroxide, 0.1 M cacodylate or collidine buffer (pH 7.1), 0.025% CaCl₂, and 0.2% NaCl for 15 min at room temperature.

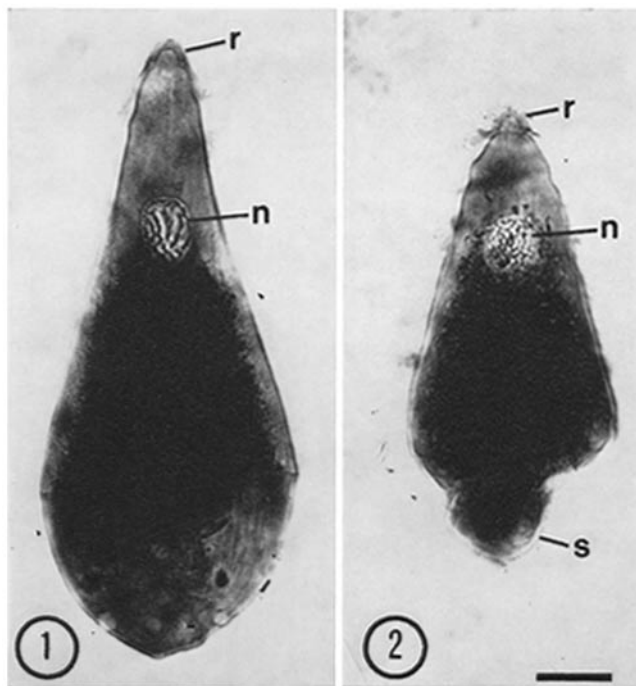
Fixed flagellates were dehydrated rapidly in an acetone series and flat-embedded in Araldite. Individual *Deltotrichonympha* and *Koruga* could be distinguished in the Araldite disc by light microscopy (see Figs. 1 and 2). Single protozoans were cut out of the Araldite and mounted on stubs for sectioning transversely or longitudinally to their anterior-posterior axis. Thick sections (0.5 μ) were cut and stained in 1% alkaline toluidine blue for light microscope observations. Thin sections (silver or silver-gold) were cut with glass knives on a Reichert OM U-2 ultramicrotome, and picked up on 200 or 300 mesh bare grids. Thin sections were stained with lead citrate (24), and 2% uranyl acetate, and examined with a Siemens Elmiskop I or a Hitachi 11-A electron microscope.

RESULTS

General Features of *Deltotrichonympha* and *Koruga*

Only a brief account of the general features of *Deltotrichonympha* and *Koruga* will be presented, since Cleveland (6-10) has already described these flagellates in detail on the light microscope level.

Deltotrichonympha and *Koruga* appear similar in many respects (Figs. 1 and 2). Both genera are



FIGURES 1 and 2 Brightfield photographs of fixed, flat-embedded *Koruga*, (Fig. 1) and *Deltotrichonympha* (Fig. 2), showing the general features of interphase organisms. Note the flagellated rostrum (*r*) at the pointed anterior end, and the large nucleus (*n*) with visible chromosomes. Pieces of ingested wood fill most of the region posterior to the nucleus. The broader posterior end of both organisms is not flagellated, and in *Deltotrichonympha* it is covered with attached spirochaete bacteria (*s*). The body flagella are not readily apparent here. Scale bar, 50 μ . $\times 200$

large hypermastigote flagellates, more or less conical in shape. *Deltotrichonympha* is typically 300–350 μ in length, while *Koruga* is longer, measuring 350–400 μ . At the pointed anterior end of both organisms is a flagellated cap, or rostrum. An elongated body is visible inside the rostrum of living cells (Fig. 3). This structure corresponds to the long and short centrioles described by Cleveland (7, 9), and will be called the centriolar apparatus in this paper. A large nucleus, containing many visible chromosomes (“chromosomal” type of interphase nucleus (17)), lies in the anterior third of the cell body (Figs. 1, 2, and 4).

Most of the body surface of *Deltotrichonympha* and *Koruga* is covered with long flagella, arranged in rows running from anterior to posterior (Figs. 3–5). The organisms swim by means of waves which begin behind the rostrum and pass posteriorly. These locomotory waves consist of

undulations in the cell cortex as well as flagellar waves (Fig. 3; reference 13).

The broader posterior end of these protozoans is not flagellated, but often amoeboid, functioning in the ingestion of wood particles which fill much of the posterior cytoplasm (Figs. 1 and 2). Such flagellates can digest cellulose, and are indispensable for the survival of the insects (20).

In addition to the size difference, *Deltotrichonympha* can be distinguished from *Koruga* by the presence of attached spirochaete bacteria on its posterior end (Figs. 1 and 2).

The general features of all the specimens of *Deltotrichonympha* and *Koruga* used in this paper, from many adult termites, conformed to this description. The appearance of these flagellates indicated that they were interphase cells, not engaged in asexual or sexual reproduction (see also Discussion).

On the ultrastructural level, *Deltotrichonympha* and *Koruga* also appear remarkably similar. Con-

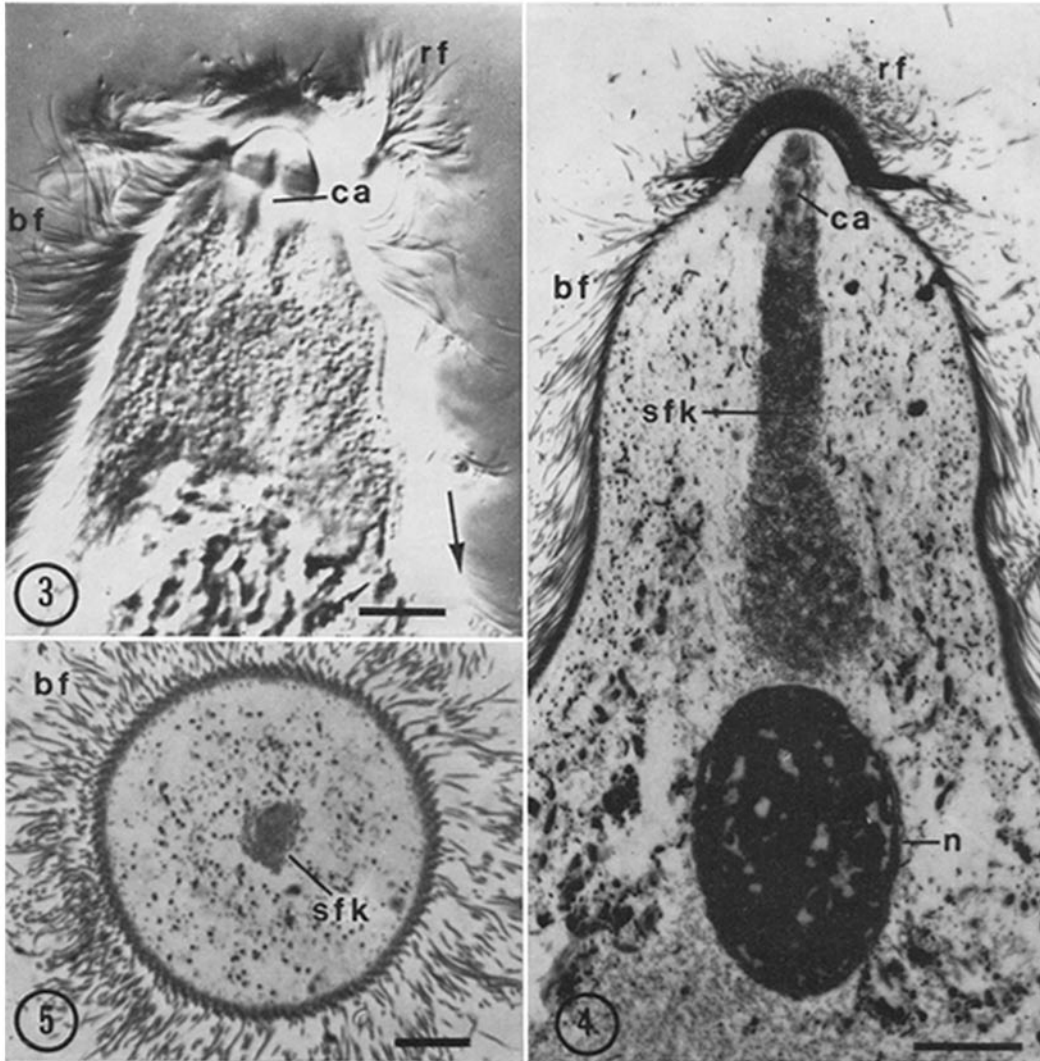


FIGURE 3 Nomarski flash photograph of the anterior end of a living *Koruga*, showing rostral flagella (*rf*), body flagella (*bf*), and the elongated centriolar apparatus (*ca*) in the rostrum. Body and flagellar waves travel posteriorly (arrow) on one side of the flagellate, but are absent on the other side. Scale bar, 20μ . $\times 580$.

FIGURE 4 Median longitudinal section (0.5μ thick) through the anterior end of *Deltotrichonympha*. Note the rostral flagella (*rf*), body flagella (*bf*), and the large nucleus (*n*) containing many condensed chromosomes. The densely-staining column of short free kinetosomes (*sfk*) extends from the centriolar apparatus (*ca*) in the rostrum to the anterior side of the nucleus. Scale bar, 20μ . $\times 720$.

FIGURE 5 Transverse section (0.5μ thick) through the anterior end of *Deltotrichonympha*, between the centriolar apparatus and the nucleus. The longitudinal rows of body flagella (*bf*) are cut transversely. The column of short free kinetosomes (*sfk*) appears as a central dense mass. Scale bar, 10μ . $\times 960$.

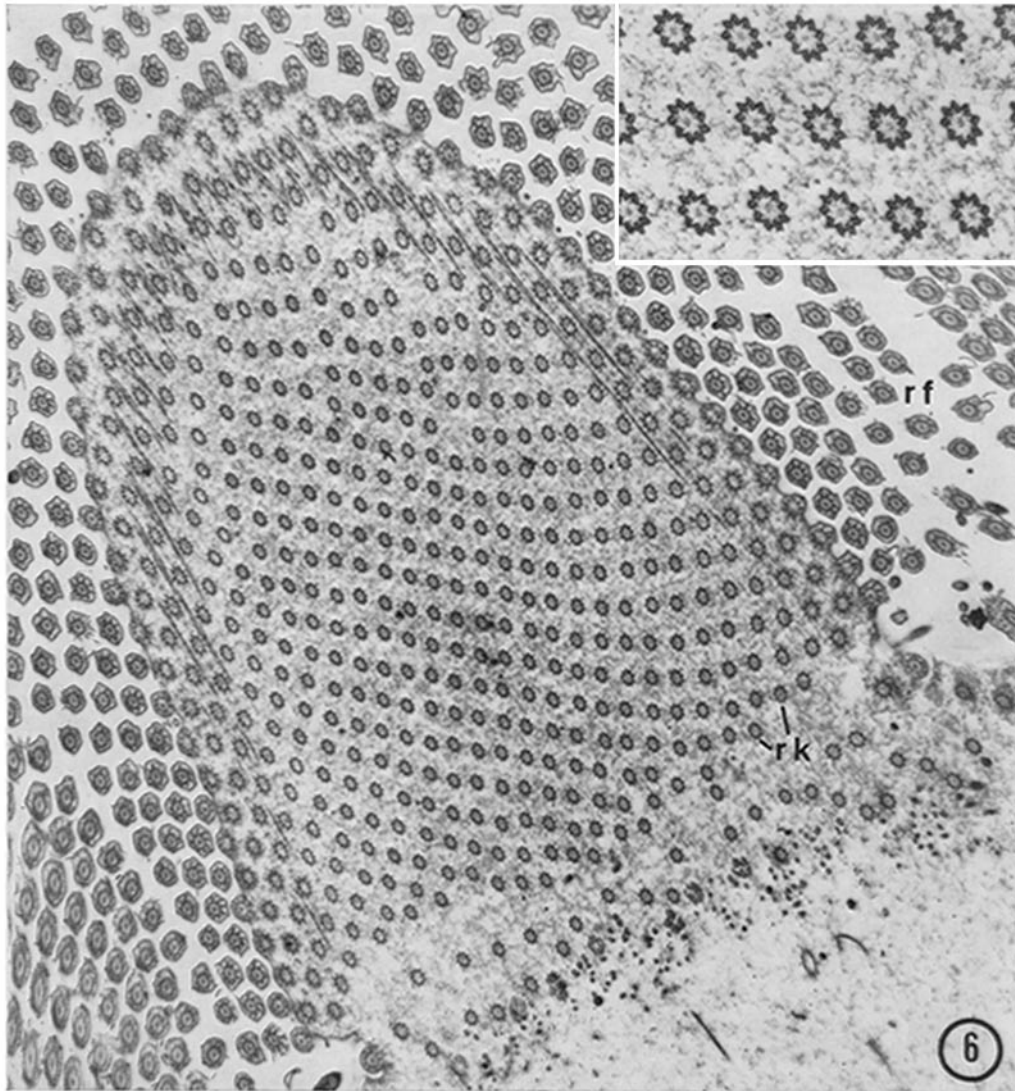


FIGURE 6 Longitudinal section grazing the side of the rostrum of *Deltotrichonympha*. The kinetosomes (*rk*) of the rostral flagella (*rf*) are cut transversely, and are arranged in a rhomboidal lattice in the outer wall of the rostrum. Thin fibers running between the distal ends of the rostral flagella kinetosomes are evident near the edge of the rostrum. $\times 12,700$. *Inset*: Higher magnification of part of Fig. 6, showing the ultrastructure of the proximal ends of the rostral flagella kinetosomes. A central cartwheel structure is visible inside the kinetosomes. $\times 39,900$.

sequently, the following electron microscope description applies to both flagellates, unless otherwise stated.

Flagellar Kinetosomes and Associated Fibers

KINETOSOMES OF ROSTRAL FLAGELLA: The kinetosomes of the rostral flagella are arranged in

intersecting diagonal rows, forming a rhomboidal lattice in the outer wall of the rostrum (Fig. 6). The kinetosomes are spaced $0.3\text{--}0.4\ \mu$ apart on the longer side of each rhomboidal unit, and $0.2\text{--}0.3\ \mu$ apart on the shorter side. They have a typical kinetosome ultrastructure: nine triplet microtubules form a ring $0.16\text{--}0.17\ \mu$ in diameter,

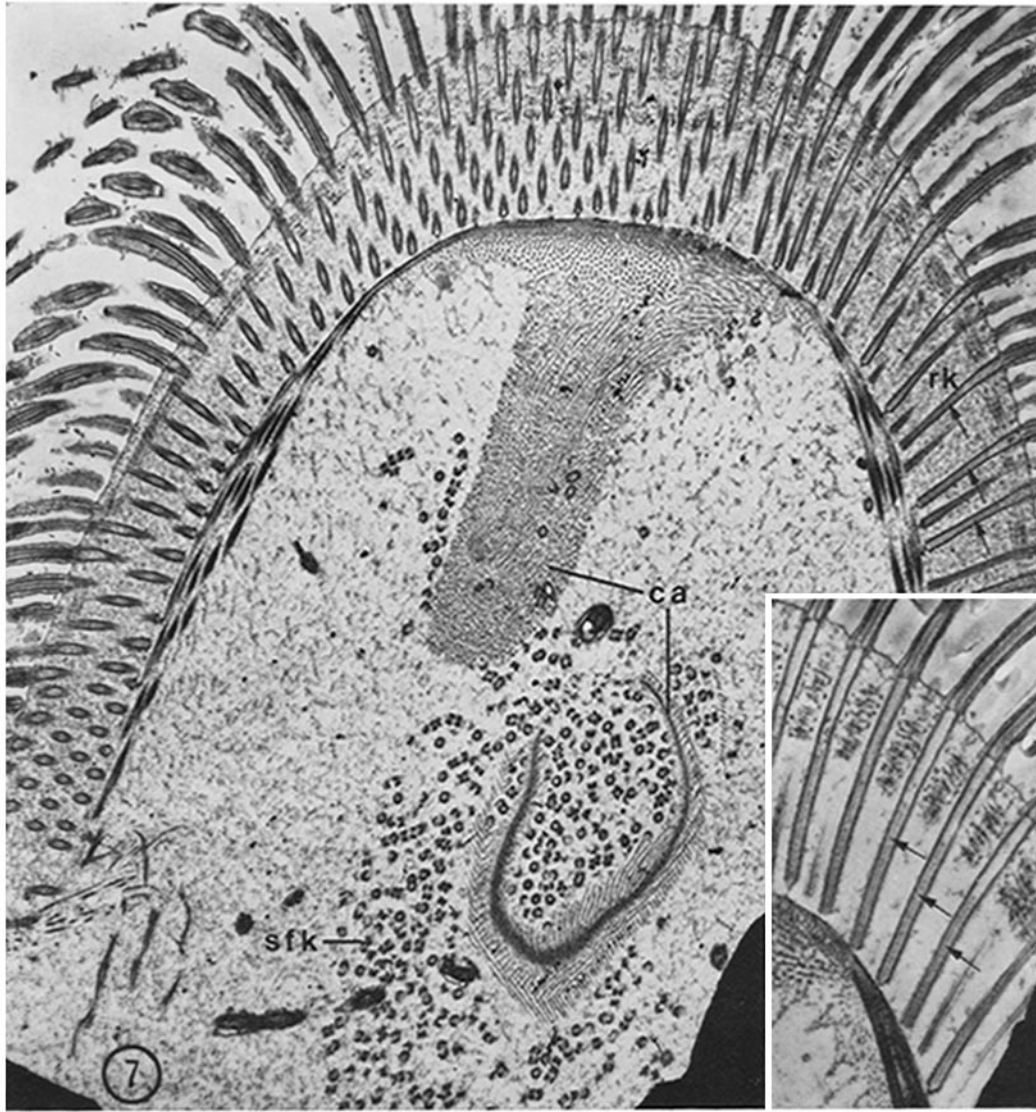


FIGURE 7 Median longitudinal section through the rostrum of *Deltotrichonympha*. The long cartwheel end of the rostral flagella kinetosomes (*rk*) is evident where these kinetosomes have been cut longitudinally (arrows). Thin fibers run between the distal ends of the rostral flagella kinetosomes. Striated fibers and dense fibers run posteriorly below the proximal ends of these kinetosomes, to which Y-shaped connections can sometimes be seen (see *Inset* also). The large granular and fibrillar bodies in the rostrum are part of the complex centriolar apparatus (*ca*). Short free kinetosomes (*sfk*), representing the anterior end of the kinetosomal column, are present in and around the lower part of the centriolar apparatus. $\times 8700$. *Inset*: Median longitudinal section through the anterior part of the rostrum of *Koruga*. Several rostral flagella kinetosomes are cut longitudinally, showing their long cartwheel structure (arrows). $\times 16,600$.

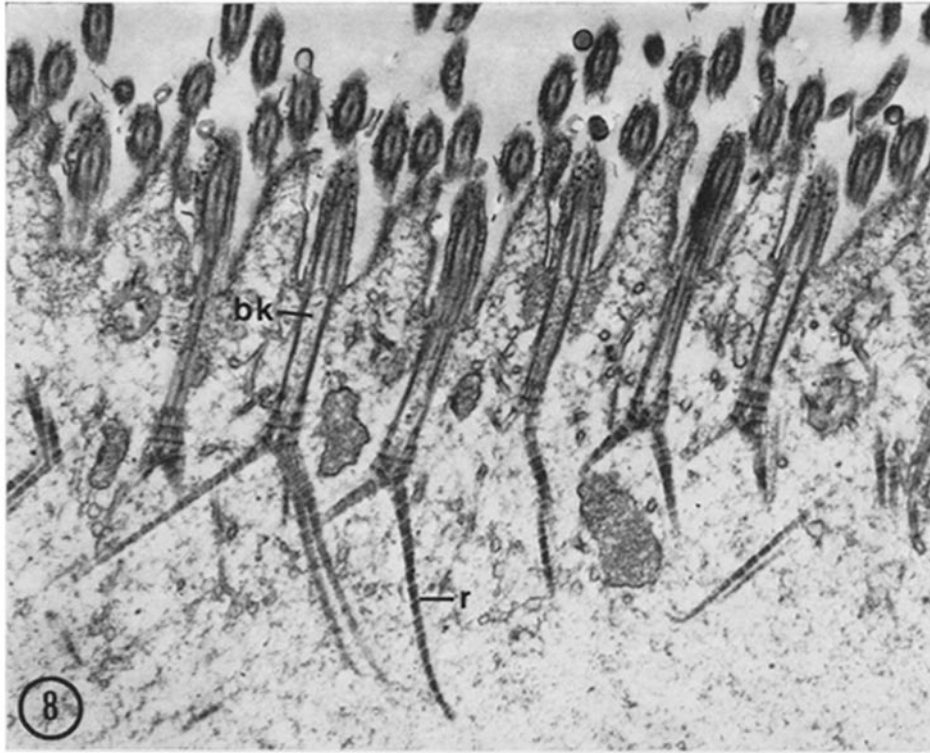


FIGURE 8 Transverse section through *Koruga*, showing the kinetosomes of the body flagella (*bk*). Branched, striated rootlets (*r*) extend downward into the cytoplasm from the proximal ends of these kinetosomes. $\times 25,200$.

with a hub-and-spokes cartwheel at the proximal end (Figs. 6 and 7). The kinetosomes are $1.2-1.5 \mu$ long at the posterior border of the rostrum, and gradually increase in length anteriorly, reaching a maximum length of $2.2-2.8 \mu$ at the apex of the rostrum (Fig. 7). The cartwheel structure of the rostral flagella kinetosomes is also long, extending 60-70% of their total length (Fig. 7)

Three kinds of fibers, all running in a posterior direction from the top of the rostrum, are associated with the rostral flagella kinetosomes. Numerous thin, coarsely striated fibers run between the distal ends of the kinetosomes near the cell surface (Figs 6 and 7). Dense fibers and finely striated fibers run just below the proximal ends of these kinetosomes, to which Y-shaped connections can be seen (Fig 7).

KINETOSOMES OF BODY FLAGELLA: The kinetosomes of the body flagella are arranged in longitudinal rows over most of the body surface. The kinetosomes within a row are spaced about

2μ apart, and adjacent rows are separated by approximately 1μ (Figs. 4, 5, 8, 13, and 14). The distance between adjacent rows of body flagella remains the same from the anterior end of the cell to the posterior end, consequently, the number of longitudinal rows of body flagella around the circumference of the flagellate increases from anterior to posterior

The body flagella kinetosomes are $0.16-0.17 \mu$ in diameter, and $0.5-0.6 \mu$ long, with little or no cartwheel structure evident (Fig. 8). Attached to their proximal ends are branched, striated rootlets which extend down into the cytoplasm.

NUMBERS OF FLAGELLATED KINETOSOMES PER CELL: The numbers of rostral flagella kinetosomes and body flagella kinetosomes in a single flagellate were estimated in the following way. From the observed spacing of each type of kinetosome, the approximate numbers of kinetosomes per unit surface area of the rostrum and body were determined. The total surface area of

the rostrum and that of the flagellated region of the body were calculated from measurements of their observed dimensions. For *Deltotrichonympha* and *Koruga*, an estimate of 4000–6000 rostral flagella kinetosomes per cell was obtained. The number of body flagella kinetosomes in a single *Deltotrichonympha* was estimated at 25,000–35,000. Because of its greater body surface area, *Koruga* was estimated to have 40,000–50,000 body flagella kinetosomes.

Centriolar Apparatus

Electron microscopy reveals that the long and short centrioles, visualized on the light microscope level by Cleveland (7, 9), do not have a typical centriolar ultrastructure (Figs. 7, 9, and 10). Instead, they appear as large, granular, and fibrillar bodies with a highly complex architecture. A detailed study of the ultrastructure and function of the centriolar apparatus in these flagellates will be presented elsewhere.²

Free Kinetosomes

One of the most striking cytological features of all interphase *Deltotrichonympha* and *Koruga* examined is the presence of a large number of free kinetosomes, without attached flagella, in the anterior cytoplasm. The free kinetosomes can be divided into two classes, short free kinetosomes and long free kinetosomes, based on their different length and spatial arrangement.

SHORT FREE KINETOSOMES: Many short free kinetosomes are concentrated in a dense column which extends from the rostrum to the anterior surface of the nucleus (Figs. 4, 5, 7, 9–13). In the rostrum, the short free kinetosomes are found mainly inside and around the lower part of the centriolar apparatus (Figs. 7 and 10). The column of short free kinetosomes is approximately round in cross section (Fig. 5), and increases in diameter from anterior to posterior (Fig. 4). It is thus similar in shape to the frustum of a cone. Measurements of the dimensions of the kinetosomal column were made from longitudinal thick sections through 13 different *Deltotrichonympha*. The kinetosomal column in these cells averaged 8 μ in diameter at its anterior end, 20 μ in diameter at its posterior end, and 70 μ in length. Compared to *Deltotrichonympha*, the kinetosomal column in *Koruga* is more slender anteriorly, and wider posteriorly. In addition, the column in *Koruga*

usually ends a short distance anterior to the nucleus.

The short free kinetosomes have a typical kinetosomal ultrastructure: nine triplet microtubules form a cylindrical wall, 0.16–0.17 μ in diameter, with a hub-and-spokes cartwheel at the proximal end (Figs. 9–12).

In most all *Deltotrichonympha* (35 of 37 cells) and most *Koruga* (20 of 30 cells) examined, from more than ten different termites, the length of the short free kinetosomes was less than their diameter. The short free kinetosomes in these flagellates are typically 0.07–0.13 μ long, with the cartwheel end extending slightly less than half this length, or 0.03–0.05 μ (Figs. 7, 9, 10, and 13). Most of this variation in length occurs between different flagellates, within any cell the length of the short free kinetosomes is remarkably uniform throughout the column (Figs. 9 and 10).

Rarely in *Deltotrichonympha*, but more commonly in *Koruga*, the short free kinetosomes were as long as, or longer than, their diameter. In five *Koruga* examined, the length of the short free kinetosomes was approximately 0.16–0.17 μ . More strikingly, in two *Deltotrichonympha* and five *Koruga*, the length of the short free kinetosomes was 0.25–0.35 μ , or about 1.5–2.0 times their diameter (Figs. 11 and 12). Nevertheless, the cartwheel end of these “longer” short free kinetosomes was similar in length to that in the typical short free kinetosomes described above (Fig. 11). So far, we have not been able to correlate the presence of the longer short free kinetosomes with the molting cycle of the termite host, since flagellates with both sizes of short free kinetosomes were found in the same termite. Attempts to correlate the size of the short free kinetosomes with the stage in the cell cycle of the flagellates have not yet been made. Again, as is the case with the typical short free kinetosomes, the length of the longer ones is fairly uniform within any given cell (Fig. 11).

Most of the free kinetosomes in the column are arranged end-to-end into chains (Figs. 9–13). The kinetosomal chains are not always straight, but sometimes bend slightly or curve. Where the kinetosomes at both ends of a chain are evident in a section, it can be seen that the chains vary in length. Up to two dozen kinetosomes have been counted in a single kinetosomal chain.

The kinetosomal chains in the unusual flagellates with longer short free kinetosomes show a definite orientation: the chains run almost perpendicular to the anterior-posterior axis of the cell

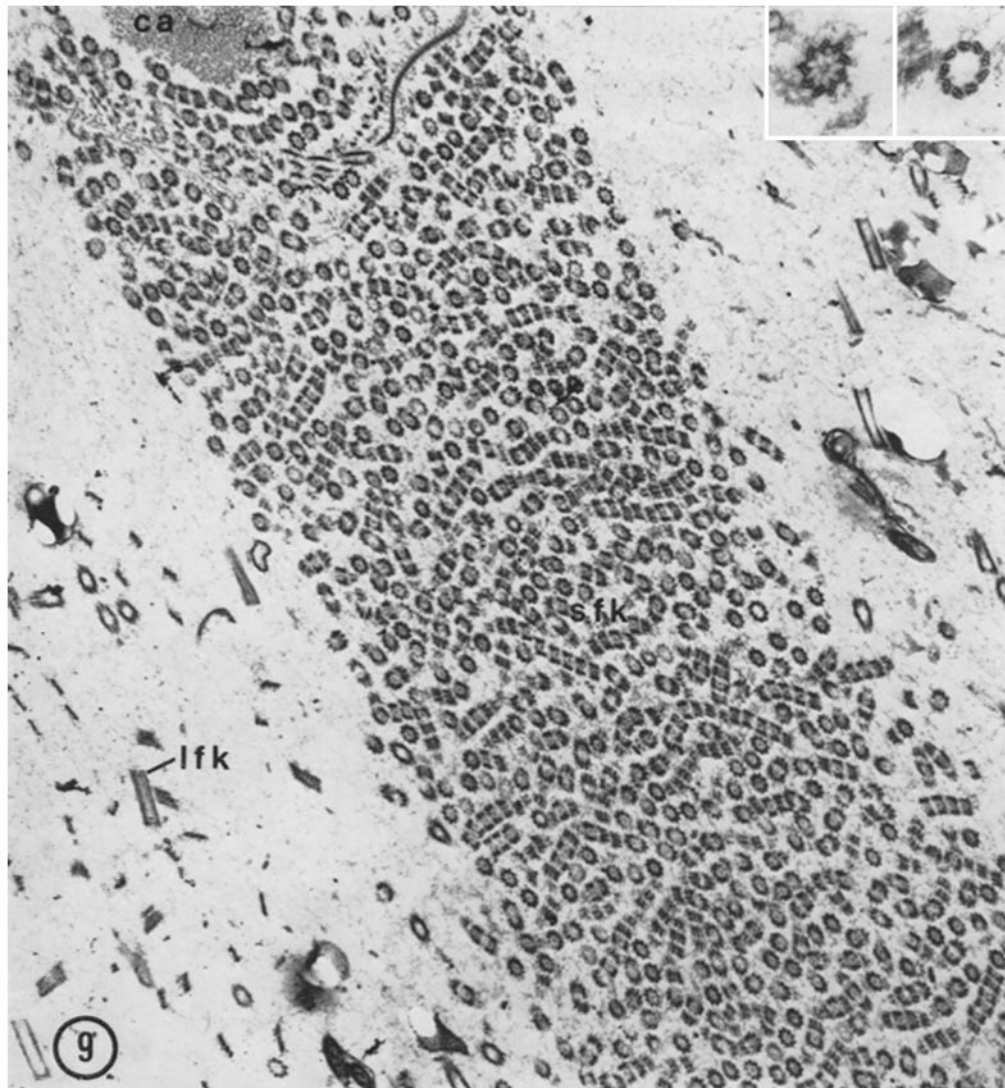
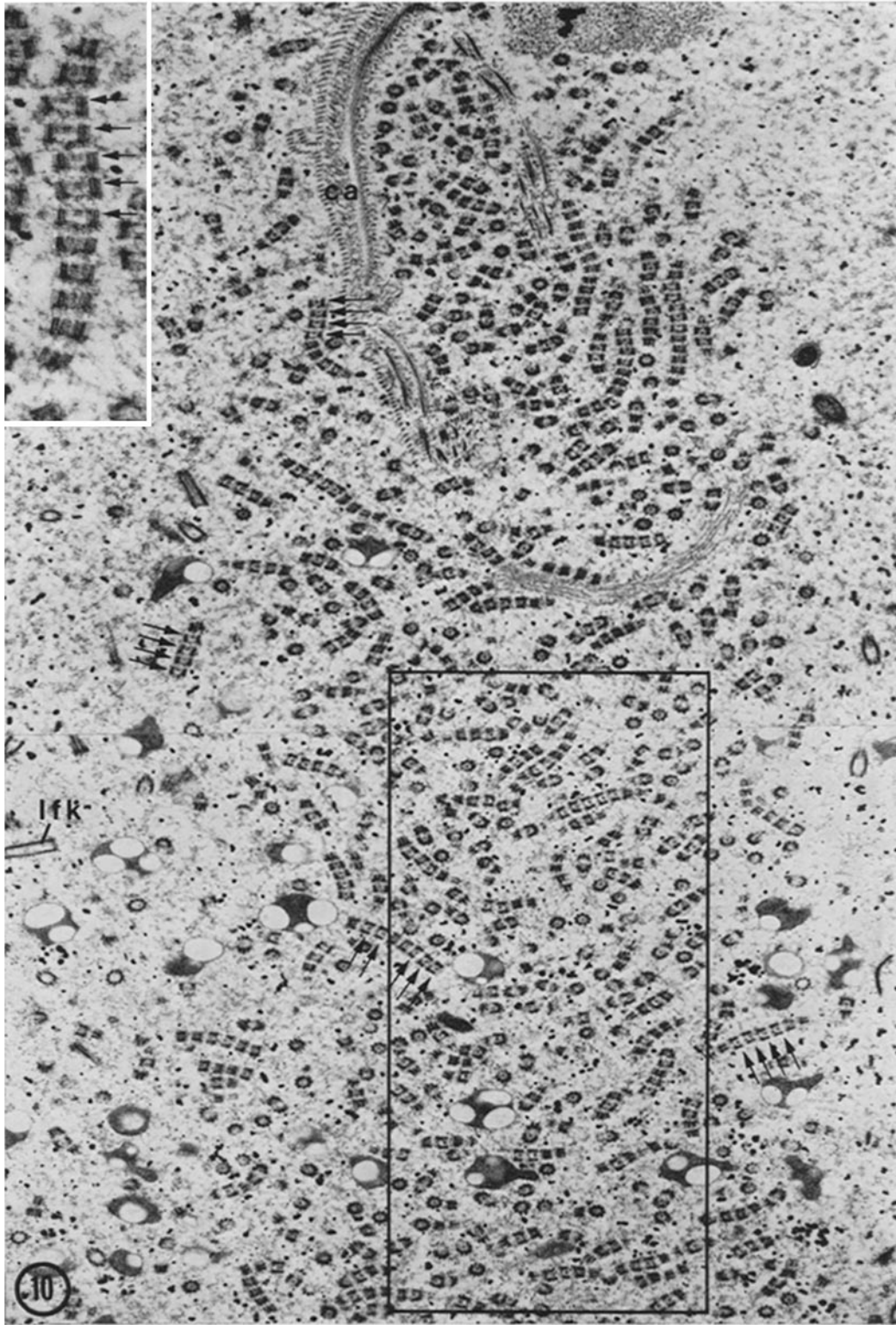


FIGURE 9 Median longitudinal section through *Deltotrichonympha*, showing the anterior end of the column of short free kinetosomes (*sfk*). The lower part of the centriolar apparatus (*ca*) is visible in the upper left of the micrograph. The free kinetosomes in the column are uniformly shorter than their diameter, and many are arranged end-to-end in chains. The packing of the kinetosomes is unusually dense in this cell (cf. Fig. 10). Long free kinetosomes (*lfk*) are scattered singly in the cytoplasm surrounding the column of short free kinetosomes. $\times 13,600$ Inset: Transverse sections through the proximal cartwheel end (left) and the distal end (right) of short free kinetosomes in *Koruga*. $\times 49,400$.

(Figs. 11 and 12), and seem to radiate from the central part of the column. A similar though much less pronounced orientation is sometimes apparent in flagellates with typical short free kinetosomes (Figs. 9 and 10).

A remarkable characteristic of the kinetosomal

chains is that they are polarized: the cartwheel ends of all the kinetosomes within any chain face in the same direction (Figs. 10 and 11). Consequently, to an observer viewing a chain from one end, all of the kinetosomes within the chain would have the same asymmetrical orientation



with respect to the direction of tilt of their triplet fibers (i.e., either clockwise or anticlockwise)

LONG FREE KINETOSOMES: A second class of free kinetosomes is found scattered singly and rather uniformly throughout the cytoplasm between the column of short free kinetosomes and the cell surface (Figs. 13 and 14). These long free kinetosomes are 0.4–0.7 μ long, similar in length to the kinetosomes of the body flagella. They have a typical kinetosomal ultrastructure, and are 0.16–0.17 μ in diameter. The length of the proximal cartwheel end in the long free kinetosomes is similar to that in the short free kinetosomes.

The long free kinetosomes were rarely found in the cytoplasm posterior to the nucleus. Since most of them appear cut transversely in cross-sections through the cells (Figs. 13 and 14), the long free kinetosomes are oriented with their long axis approximately parallel to the anterior-posterior axis of the cell. The long free kinetosomes were found in all *Deltotrichonympha* and *Koruga* examined, including those flagellates with longer short free kinetosomes.

NUMBERS OF FREE KINETOSOMES PER CELL: To gain a clue about the fate of the free kinetosomes, a preliminary attempt was made to estimate their number per cell. The object was to obtain a rough idea of the approximate number of free vs. flagellated kinetosomes in a single organism. These calculations were made mainly on *Deltotrichonympha* with typical short free kinetosomes.

The number of short free kinetosomes in a single cell was estimated in the following way. First, the total volume of the kinetosomal column in *Deltotrichonympha* was calculated to be 10,000 μ^3 , based on its shape and average dimensions described above. The fraction of this volume occupied by short free kinetosomes (the volume fraction of kinetosomes) was determined by calcu-

lating the fraction of a given area of the column occupied by short free kinetosomes, in longitudinal thin sections (Fig. 10). The packing of the kinetosomes is fairly uniform throughout the column of any given cell. Measurements on kinetosomal columns in ten different *Deltotrichonympha*, and several *Koruga*, yielded typical volume fractions of 0.10–0.15 for the short free kinetosomes. Occasionally, flagellates with a greater density of kinetosomes were observed (Fig. 9). Multiplying the volume fraction times the total volume of the column gave the volume occupied by short free kinetosomes in the column. This volume was then divided by the average volume of one short free kinetosome (i.e., 0.002 μ^3 , using a length of 0.1 μ and a diameter of 0.16 μ).

With this counting procedure, we obtained a minimum-maximum estimate of 500,000–750,000 short free kinetosomes in the column of a single *Deltotrichonympha*. No attempt was made to compare the numbers of longer vs. typical short free kinetosomes in different flagellates.

The number of long free kinetosomes per cell was estimated by the same procedure used to count the short free kinetosomes. Since the long free kinetosomes are distributed almost entirely in the cytoplasm anterior to the nucleus, only the volume of this part of the cell was used in the calculation. This volume is approximately 100,000 μ^3 in *Deltotrichonympha*. Transverse thin sections through several *Deltotrichonympha* at various levels between the rostrum and the nucleus were used to determine the fraction of this volume occupied by long free kinetosomes (Fig. 13). Typical values for the volume fraction ranged from 0.007 to 0.012. The average volume of one long free kinetosome was estimated at 0.010 μ^3 , using a length of 0.5 μ and a diameter of 0.16 μ . The resulting calculations gave an estimate of 70,000–120,000 long free kinetosomes per *Deltotrichonympha*.

FIGURE 10 Median longitudinal section through *Koruga*. The anterior end of the column of short free kinetosomes begins in the lower part of the centriolar apparatus (*ca*). Most of the short free kinetosomes are grouped end-to-end in chains. Within a chain, the cartwheel ends of all the short free kinetosomes face in the same direction (arrows). Note that the cartwheel end of the short free kinetosomes extends about half of their length (see also *Inset*). The total length of the short free kinetosomes is less than their diameter, and does not vary appreciably within a chain, or between different chains. The fraction of the outlined area occupied by short free kinetosomes is 0.10, giving a volume fraction estimate of 0.10 for the short free kinetosomes in this column (see text). Long free kinetosomes (*lfk*) are scattered singly in the surrounding cytoplasm. $\times 13,900$. *Inset*: Higher magnification of a chain of short free kinetosomes shown in Fig. 10. The cartwheel ends of all the individual kinetosomes within the chain face toward the top of the micrograph (arrows). $\times 38,000$

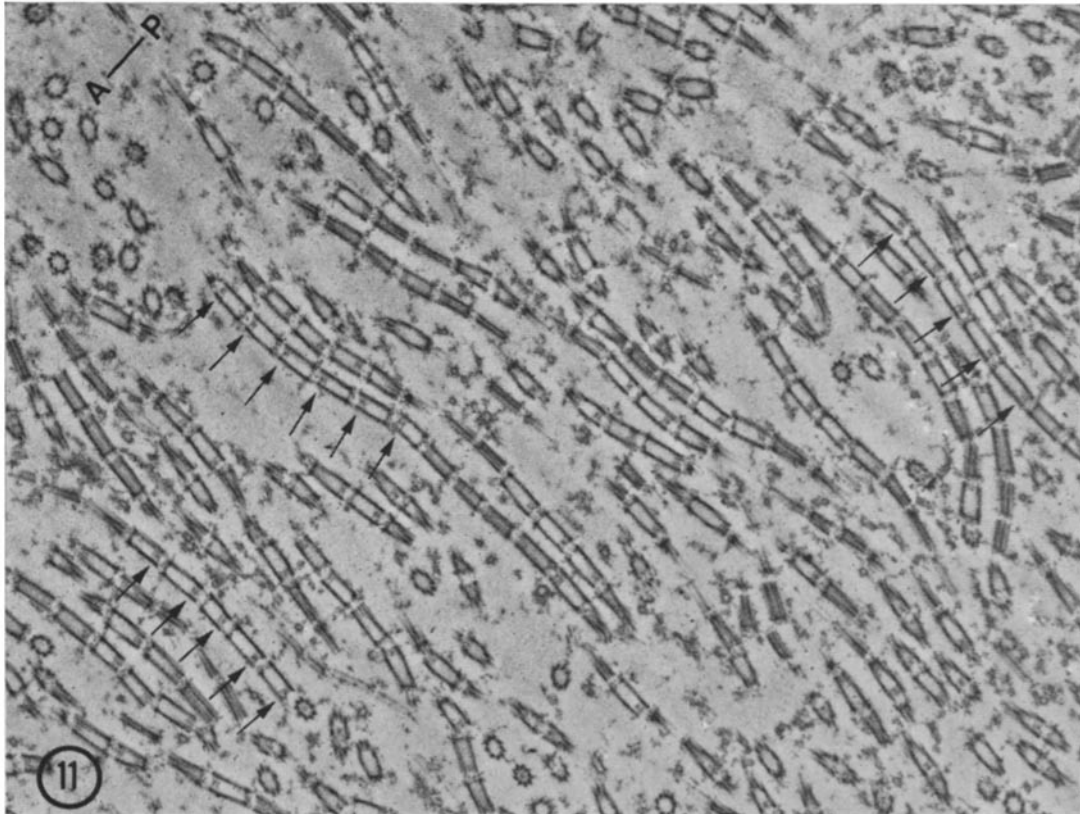


FIGURE 11 Longitudinal section through a column of "longer" short free kinetosomes in *Koruga*. These kinetosomes are uniformly longer than the short free kinetosomes in typical cells, yet have a similar cartwheel end (cf. Figs. 9 and 10). The chains of longer short free kinetosomes, like those of typical short free kinetosomes, are polarized with respect to the proximal cartwheel ends (arrows). However, the chains of longer short free kinetosomes are uniformly oriented at right angles to the anterior-posterior axis of the cell (A-P). $\times 15,900$

NUMBERS OF FREE VS. FLAGELLATED KINETOSOMES PER CELL: Table I summarizes the data on the numbers of free and flagellated kinetosomes in a single *Deltotrichonympha*. The total number of free kinetosomes per cell is 570,000–870,000 (500,000–750,000 short free kinetosomes, plus 70,000–120,000 long free kinetosomes). In contrast, the total number of flagellated kinetosomes per cell is only 29,000–41,000 (4000–6000 rostral flagella kinetosomes, plus 25,000–35,000 body flagella kinetosomes). The less extensive observations on *Koruga* indicate a similar difference between the numbers of free and flagellated kinetosomes per cell.

DISCUSSION

One's first reaction to the remarkable concentration of free kinetosomes described here is: Why?

What is their significance? To answer these questions, the fate of the free kinetosomes must be followed through the complete life-cycle of the flagellates. Unfortunately, this has not been possible to do in the present study, because only non-reproductive, interphase cells were used. However, several possible explanations for these findings can be listed, along with directions for future research.

The most obvious possibility is that the free kinetosomes are related to morphogenetic changes that occur at cell reproduction. Both asexual and sexual reproduction have been reported for *Deltotrichonympha* and *Koruga* (6–10). The sexual cycle of these flagellates from *Mastoterme*s is supposed to be similar to that of *Barbulanympha* from *Cryptocercus*, and likewise correlated with the molting period of the insect host (4, 8–10).

The asexual cycle of *Deltotrichonympha* and

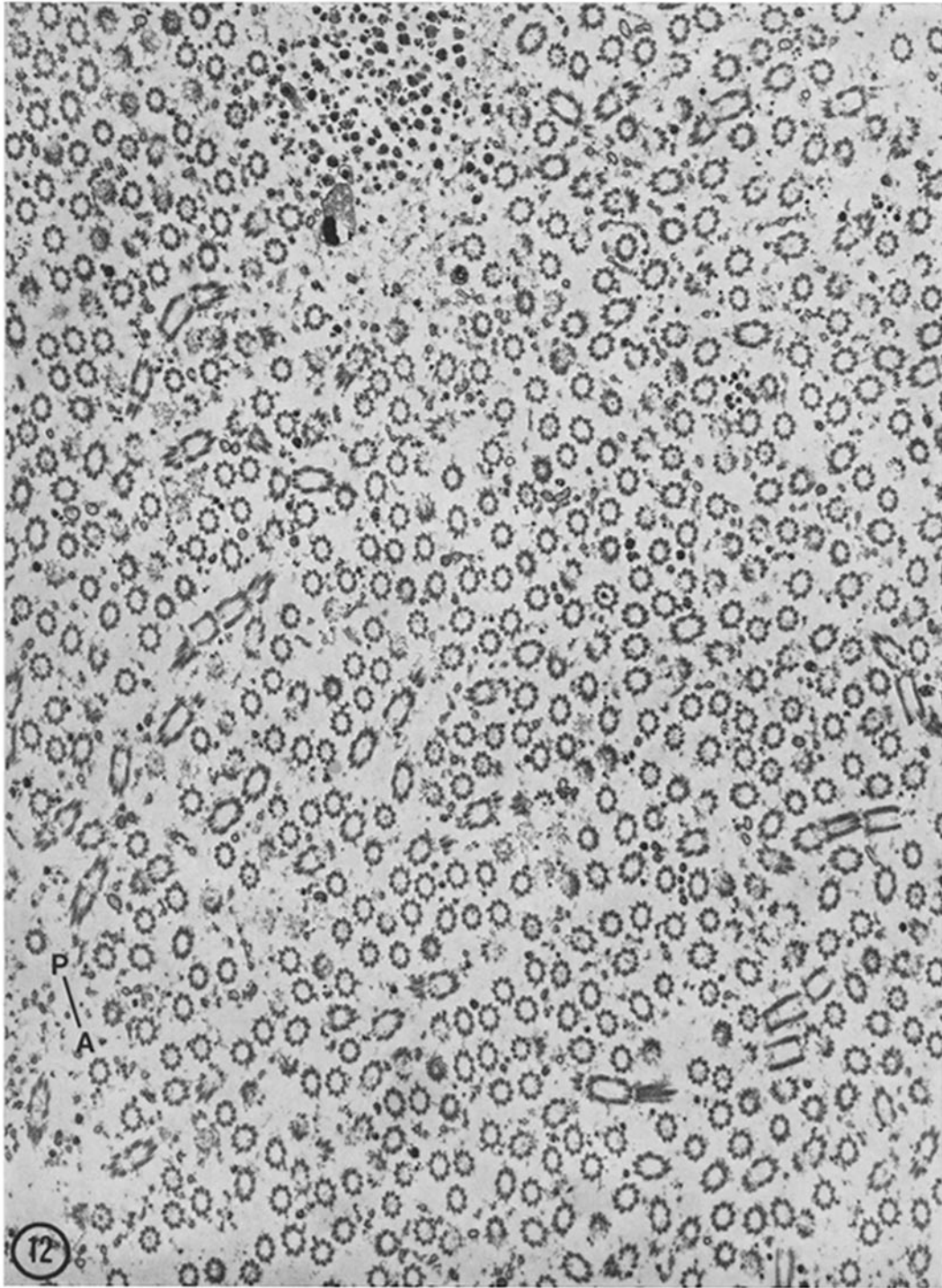


FIGURE 12 Longitudinal section through a column of "longer" short free kinetosomes in *Koruga*. Most of the kinetosomal chains are cut transversely, and run perpendicular to the anterior-posterior axis of the cell (A-P). $\times 17,500$.

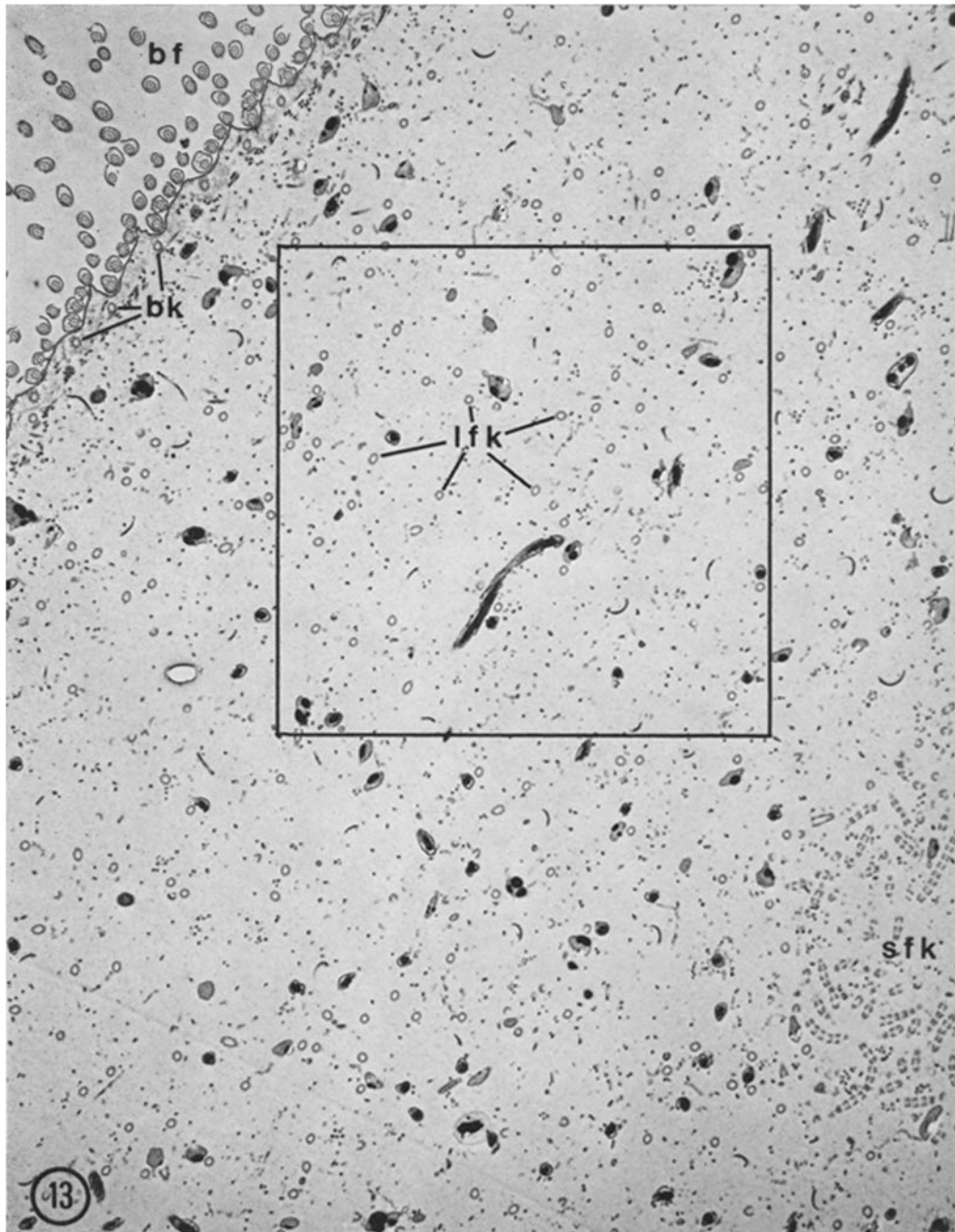


FIGURE 13 Transverse section through the anterior end of *Deltotrichonympha*, between the centriolar apparatus and the nucleus. Long free kinetosomes (*lfk*) are scattered singly and rather uniformly throughout the cytoplasm between the column of short free kinetosomes (*sfk*) and the cell surface. Most of the long free kinetosomes are sectioned transversely, showing their orientation parallel to the anterior-posterior axis of the cell. The fraction of the outlined area occupied by long free kinetosomes is 0.011 in this section, resulting in a volume fraction estimate of 0.011 for the long free kinetosomes in this cell (see text). Rows of body flagella (*bf*) and body flagella kinetosomes (*bk*) are evident at the cell surface. $\times 7500$.

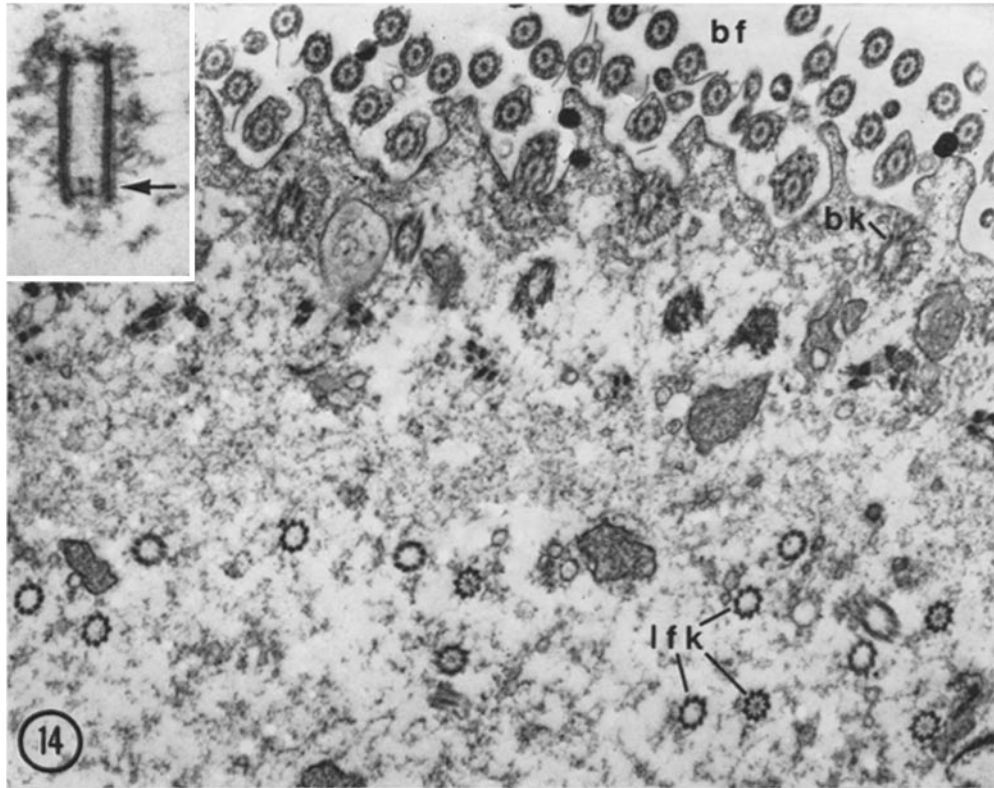


FIGURE 14 Cross-section through the anterior end of *Koruga*, at the cell surface. Long free kinetosomes (*lfk*) are cut transversely, showing their typical kinetosomal ultrastructure. A cartwheel structure is evident in some long free kinetosomes. Rows of body flagella (*bf*) arise from the body flagella kinetosomes (*bk*) at the cell surface. $\times 26,000$. *Inset*: One long free kinetosome sectioned longitudinally (*Koruga*). Note the relatively short cartwheel end (arrow). $\times 45,800$.

TABLE I
Numbers of Free vs Flagellated Kinetosomes Per Cell

Kinetosome type	Number per cell (<i>Deltotrichonympha</i>)
Flagellated	
Rostral	4000-6000
Body	25,000-35,000
Total.	29,000-41,000
Free	
Short	500,000-750,000
Long	70,000-120,000
Total.	570,000-870,000

Koruga may also be correlated with the molting period of the insect, as is evidently the case for flagellates from other termites (1). However, unlike cell division in complex hypermastigote flagellates from other termites, *Deltotrichonympha*

and *Koruga* reportedly lose all flagella before mitosis, and divide in an amoeboid state (6, 7, 9, 21). Such temporary loss of flagella at cell division is not unusual in the protozoa; it commonly occurs in free-living algal flagellates (28), as well as in some simpler hypermastigote flagellates from termites (21).

The reproductive cycles of *Deltotrichonympha* and *Koruga*, as described by Cleveland, thus present no remarkable feature that might explain the unique presence of so many free kinetosomes in these organisms. Indeed, previous electron microscope studies of simple and complex hypermastigote flagellates from other termites and from *Cryptocercus* have never revealed any free kinetosomes comparable to those found in *Deltotrichonympha* and *Koruga* (2, 18, 19).

The sheer numbers of free kinetosomes also argues against any simple role for them in asexual

or sexual reproduction. The total number of flagellated kinetosomes per *Deltotrichonympha* was estimated to be 29,000–41,000, whereas the total number of free kinetosomes per cell is probably 570,000–870,000 (Table I). Thus, even if a mitotic cell lost all the kinetosomes associated with the body and rostral flagella when it lost these flagella, the number of free kinetosomes present would be many times greater than the number of new kinetosomes needed to equip both daughter cells with complete sets of body and rostral flagella.

Another possible interpretation of the free kinetosomes is that they may be related to morphogenetic events taking place during the interphase period of the cell cycle. For example, the body flagella kinetosomes may have a short lifetime relative to the long generation time assumed for termite flagellates (1), and be continually lost and replaced by the “pool” of free kinetosomes. However, one would wonder why free kinetosomes have not been found in other termite flagellates, with presumably similar generation times. Nevertheless, the similarity in length and spatial orientation between the body flagella kinetosomes and the long free kinetosomes, as well as their close proximity at the cell surface, indicates a possible relationship between these kinetosomes.

The relationship between the two types of free kinetosomes found in a single cell is another unsolved problem. For instance, we do not know whether the short and long free kinetosomes arise independently and are two unrelated populations, or whether they share a common origin and simply represent different developmental stages. The finding of occasional flagellates with longer short free kinetosomes intermediate in length between typical short free kinetosomes and long free kinetosomes, and the uniform length of the cartwheel end in all size classes of free kinetosomes, suggests that short free kinetosomes may become long free kinetosomes by growth at the distal end. If so, then this growth must occur synchronously throughout the kinetosomal column, since the length of the longer short free kinetosomes is remarkably uniform within a given cell. In addition, the infrequent occurrence of flagellates with longer short free kinetosomes indicates that any kinetosomal growth must take place during a relatively short period of the cell cycle.

One of the most intriguing aspects of the free kinetosomes is the end-to-end arrangement of the short free kinetosomes into polarized chains, in

which the triplet fibers of all the kinetosomes in a chain are tilted in the same orientation (clockwise or anticlockwise). Initially, this arrangement suggested a replication pattern for kinetosomes, whereby already formed kinetosomes could exert a direct template action on formation of new ones. Such a rubber-stamp template model of centriole duplication, although attractive, has never been supported by electron microscope studies of centriole formation (15). However, our recent work on reproductive stages of *Deltotrichonympha* and *Koruga*, obtained from recently-molted nymphs of *Mastotermes*, has revealed a different mode of origin of the free kinetosomes, that occurs before they become arranged end-to-end in chains¹ (27).

It therefore appears that the kinetosomal chains in interphase cells do not present stages in kinetosome formation. Rather, these kinetosomes probably represent an accumulation of “young” kinetosomes, arrested in an early stage of growth, and stored end-to-end in chains. The reason for this unique pattern of kinetosomal storage, if indeed this is the case, is not readily apparent. Perhaps this arrangement is related to the ultimate destination of these kinetosomes, which may be the ordered rows of body and/or rostral flagella kinetosomes.

It should be clear that an investigation of a complete series of reproductive stages of *Deltotrichonympha* and *Koruga*, from termites at different periods in the molting cycle, should provide answers to many of the questions concerning the behavior, function, and origin of the free kinetosomes. In addition, the unparallelled concentrations of free kinetosomes in these flagellates should provide useful material for large-scale isolation of kinetosomes for biochemical studies.

I am grateful to Professor Adrian Horridge, Department of Behavioural Biology, Australian National University, for generously providing laboratory facilities and encouragement, and the staff of the John Curtin School of Medical Research, Canberra, for the use of their excellent electron microscope facilities.

R. E. Fox, of the Forest Research Institute, kindly supplied the termites from Darwin, Northern Territory.

I would also like to thank Gary Grimes, of the Zoology Department, Indiana University, for his helpful criticism of the manuscript.

A preliminary account of this work was presented at the 1971 American Society for Cell Biology meeting in New Orleans (27).

Most of this work was done at the Australian

National University, Research School of Biological Sciences, Canberra, under a Visiting Research Fellowship awarded to the author. The work was also supported in part by a National Science Foundation Grant GB-8714

Received for publication 28 December 1971, and in revised form 28 February 1972.

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