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SHORT COMMUNICATION

ELECTON MICROSCOPIC OBSERVATIONS ON DIVISION OF KINETOPLASTS IN *TRYPANOSOMA GAMBIENSE*

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The kinetoplast of the Trypanosomatidae is a self-duplicating particle close to the base of the flagellum. This organella has been considered to be a differentiated part of the large mitochondrion found in these flagellated protozoa (Steinert, M. 1960). Employing Feulgen staining and autoradiography with tritiated thymidine, it was clearly shown that the kinetoplast contains DNA (Steinert et al., 1958, Inoki, S. and T. Ono, 1966, 1967, 1968). Light microscopy showed that the kinetoplast divides into two daughter kinetoplasts by binary fission, and its division always takes place before nuclear division in a replicating cell (Steinert, M. et G. Steinert, 1962). The present paper describes the stages in duplication of the kinetoplast observed with an electron microscope.

The Wellcome strain of *Trypanosoma gambiense* was used throughout this series of experiments[1]. Mice were infected with the organisms and their blood was removed 3–4 days after infection. Blood containing trypanosome cells was fixed at 4 C for one hour in 0.013 M phosphate buffer (pH 7.4) containing 2.5% glutaraldehyde. Trypanosome cells were separated from blood cells by gentle centrifugation at 2,000 rpm for 15 min in a swinging type centrifuge. The protozoa cells formed a film on the top of the packed blood cells. The film was removed with a fine glass rod and washed for one hour with phosphate buffer containing 0.25 M sucrose, changing the buffer four times. Then it was stored at 4 C overnight in the same isotonic buffer. The materials were post-fixed at 4 C for one hour with 1.5% osmium tetraoxide in isotonic buffer, and then washed once with distilled water. After dehydration with a series of increasing concentrations of alcohol, materials were embedded in Epon 812 and sections were further stained with uranyl acetate, followed by lead citrate (Reynolds, E.S. 1963). Micrographs were made in a Hitachi 11 B electron microscope.

On examination of many sections, we occasionally found kinetoplasts in the dividing stage and from these micrographs, the duplication of the kinetoplast in *T. gambiense* was studied. In certain cells, two basal bodies (or blepharoplasts) were observed in the neighborhood of the kinetoplast within a single cell (Figs. 1 and 4). It seems, therefore, that duplication of the basal body occurs before the

1 This strain was given by courtesy of Dr. Max C. McCowen, The Eli Lilly Research Laboratories, Indianapolis, Indiana, U.S.A.
The stock arrived on Feb. 14, 1956, and since then it has been maintained in mice in our laboratory.

FIGURES 1. to 10. Electron micrographs of *Trypanosoma gambiense* in its form in the blood stream. The scale in each figure shows 0.5 μ length.
bb, basal body; fl, flagellum; K, kinetoplast; dk, daughter kinetoplast; N, nucleus

FIGURE 1. *Section of two basal bodies (bb) close to a kinetoplast.* One is a lateral, and the other a vertical section.

FIGURE 2. *Section of an elongated kinetoplast.* The arrows show cytoplasmic inpocketing (see text).

FIGURES 3. and 4. *Sections of dividing kinetoplasts.* Two basal bodies are also seen in Fig. 4.

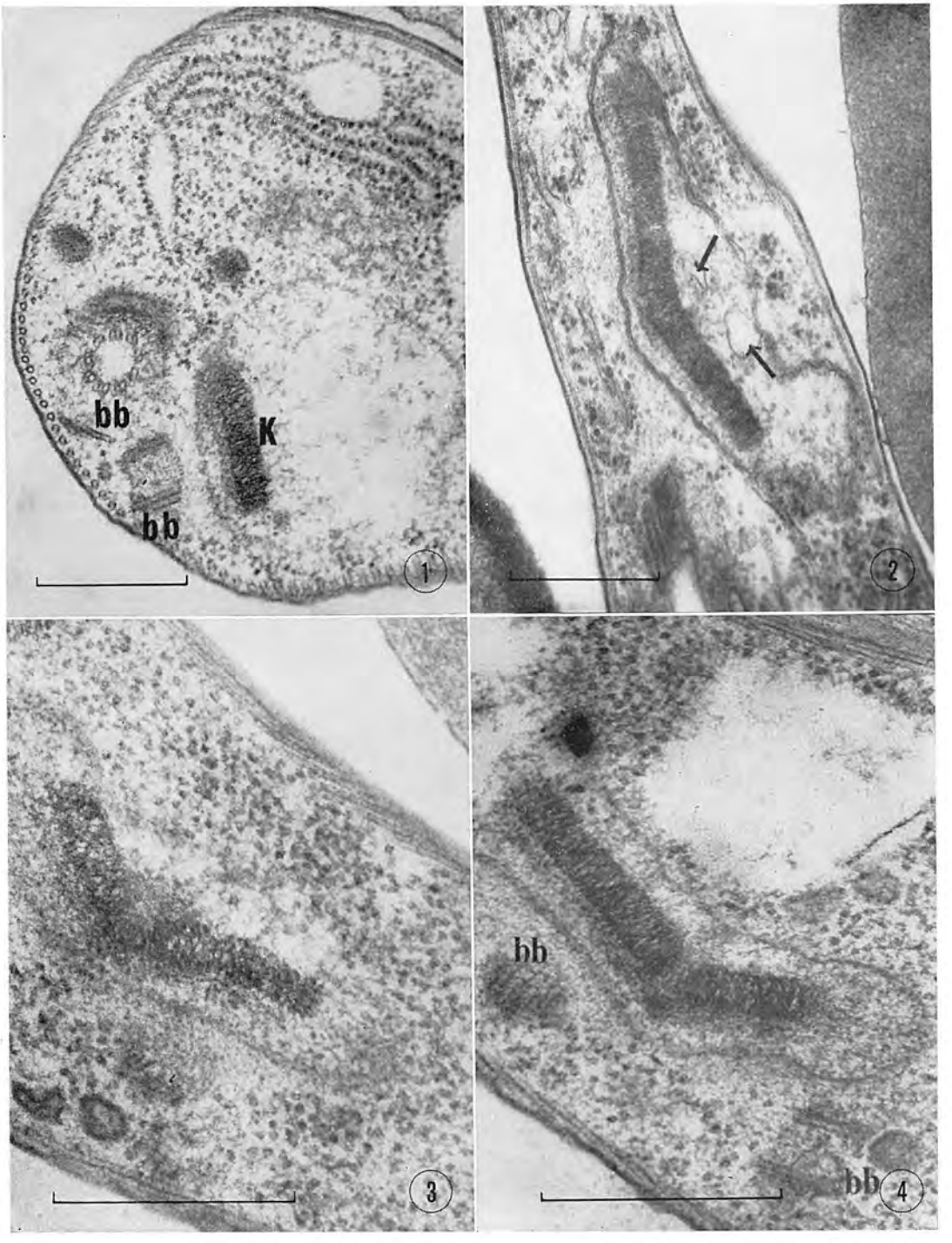

bb
K
bb
1
2
3
bb
bb
4

FIGURE 5. *Section of two daughter kinetoplasts after separation.*

A. One of the kinetoplasts [on the left] has an amorphous structure: the fibrils are indistinct.

B. Both kinetoplasts show a fibrous structure.

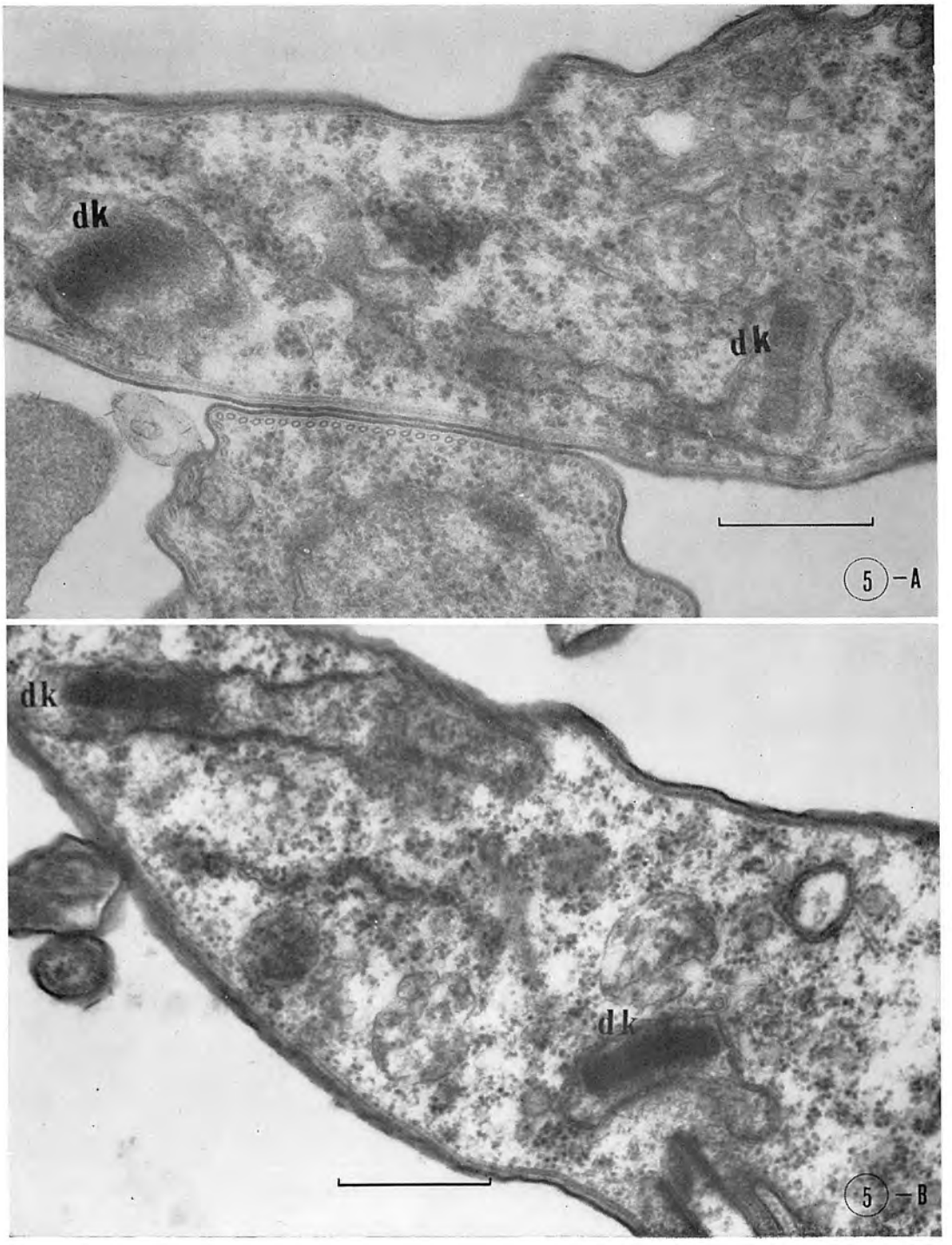

dk
dk
5 -A
dk
dk
5 -B

FIGURE 6. *Section of a daughter kinetoplast, in which fibrils seem to be reappearing in the amorphous structure.*

FIGURE 7. *Section of a kinetoplast containing thicker fibrils than those seen in Figs* 5. *and* 6. This is presumably a later stage in which condensation of fibrils is taking place. In this stage, the rod-shaped kinetoplast may be formed.

FIGURE 8. *Section of a typical rod-shaped kinetoplast.* In most sections, the kinetoplast is of this type, in which the kinetoplast is in a non-duplicating stage.

FIGURE 9. *Section of T. gambiense treated with p-rosaniline.* A slender fibrous structure can be seen between two daughter kinetoplasts. The arrows show material from the kinetoplast fragmented by the dye.

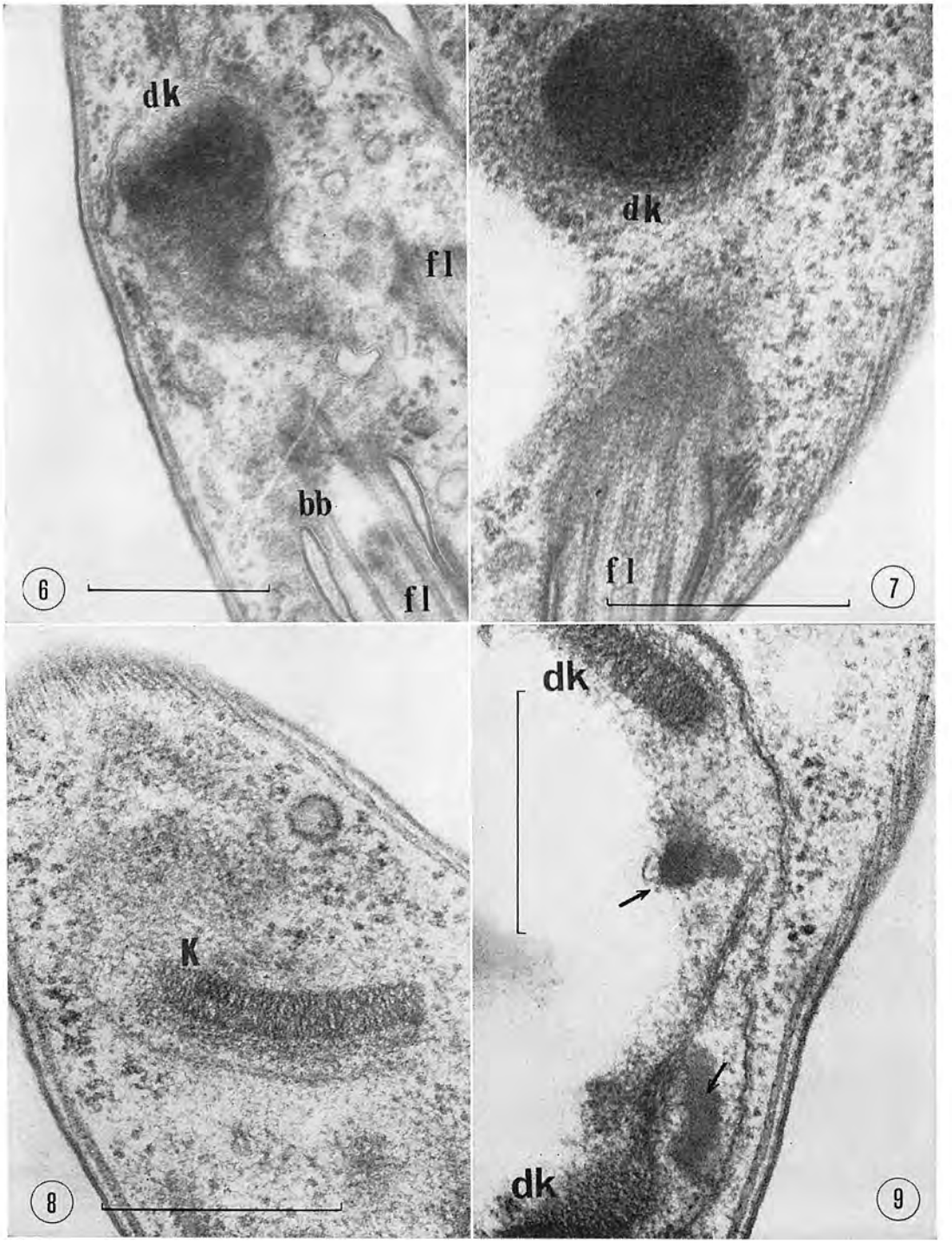
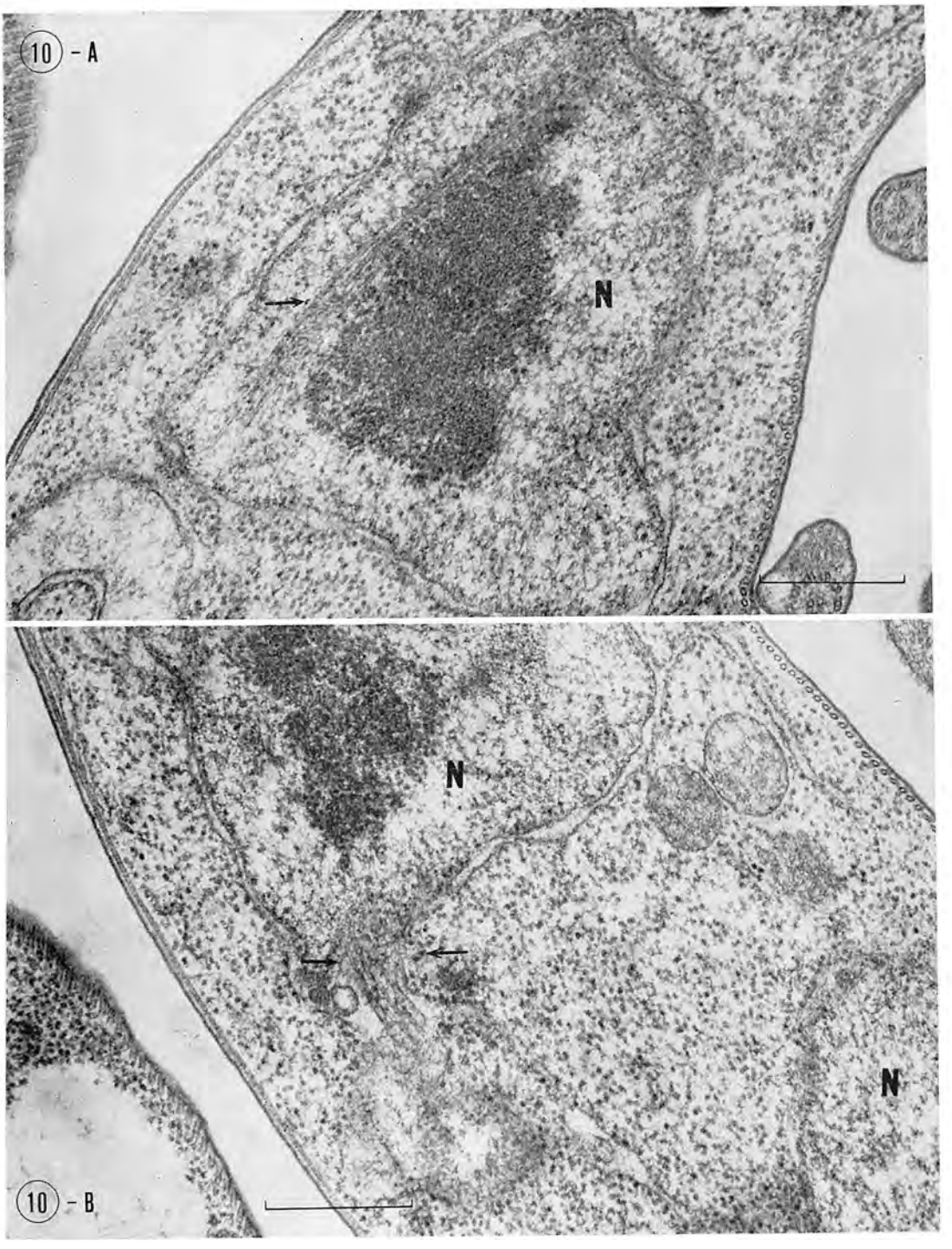

dk
fl
bb
fl
6
dk
fl
7
K
8
dk
dk
9

FIGURE 10. *Serial sections of paired daughter nuclei.*

A. The microtubules are visible in one of the two daughter nuclei (arrow).

B. Two daughter nuclei are seen. The microtubules seen in Fig. 10A are not visible in this section, but a lateral section of a bundle of microtubules can be seen at the peripheri of the nucleus (arrows). Microtubules from the nucleus, perhaps connecting two daughter nuclei, can also be distinguished.


10 - A
N
10 - B
N
N

kinetoplast begins to divide. The axes of the two daughter basal bodies were always perpendicular to each other (see bb in Figs. 1 and 4). This appearence bears strong resemblance to the centrosome in other organisms (Renaud, F.L., and H. Swift, 1964). The role of the basal body also seems comparable to the general role of the centrosome (Johnson, U.G. and K. R. Porter, 1968).

Fig. 2 shows an elongated kinetoplast cut along its axis, which is bent in the middle. The arrows indicate the cytoplasmic inpocketing. This inpocketing is believed to be a prelude to division of mitochondria in other species of trypanosomes (*T. vivax*), in which the continuity of the kinetoplast envelope with the mitochondria was demonstrated (Rudzinska, M.A. and K. Vickerman, 1968). The division of the kinetoplast proceeds by incision at the bend in the elongated kinetoplast (Figs. 3 and 4) and finally the two daughter kinetoplasts separate completely (dk, Fig. 5). The separation of the daughter basal bodies occurred before the kinetoplast, which has just divided, begins to separate. It is known that the kinetoplast in trypanosomes contains electron-dense fibers. Our micrographs show that these fibers persist during division of the kinetoplast. However, one of the kinetoplasts in Fig. 5A which had just divided shows a somewhat amorphous structure. Fig. 6 also shows an amorphous kinetoplast. Subsequently, thicker fibers appear in this amorphous kinetoplast (Fig. 7) and then the kinetoplast changes into a normal rod-shaped kinetoplast before cell division occurs (Figs. 5B and 8). The amorphous stage might be a stage of active-synthesis, and if so, at this stage the kinetoplast may contain a double-amount of kinetoplastic material which will separate in the next division.

Fig. 9 is a micrograph obtained after elimination of the kinetoplast by treatment of the same strain of trypanosome with *p*-rosaniline (Inoki, S., Y. Ozeki and T. Ono 1968). A slender fibrous structure can be seen between two separating daughter kinetoplasts. This could be a stage in the normal division of the kinetoplast, or an abnormality in the kinetoplast due to treatment with the dye.

In many species of protozoa microtubules are observed during nuclear division, inside or outside the nuclei depending upon the species (Roth, L.E., and O. T. Mimick, 1961; Roth, L.E. and Y. Shigenaka, 1964; Inaba, F. and Y. Sotokawa, 1968). The appearance of these microtubules is believed to be correlated with nuclear division of the protozoa. In the strain of *T. gambiense* used in this study, microtubules were observed within the nuclei. Figs. 10 A and 10 B show two serial sections of a dividing organism. The two daughter nuclei may be connected by the microtubules (Fig. 10 B), and one of the nuclei also contains microtubules (indicated by the arrow in Fig. 10 A). A similar observation was made in our laboratory on *T. evansi* (Murata, unpublished). In contrast, no such structure was found in relation to division of the kinetoplast.

Thus we found that division of the kinetoplast takes place by elongation, bending at the middle and incision of the kinetoplast. Its fibrous structure is retained during the division, but disappears immediately after separation of the two daughter kinetoplasts. The fibrous structure subsequently reappears in the daughter kinetoplasts, and the rod-shaped kinetoplast is formed. A correlation between division of the basal body and that of the kinetoplast was also confirmed.

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