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SHORT COMMUNICATION

POSSIBLE CONVERSION OF AXONEMAL MICROTUBULES TO PELLICULAR MICROTUBULES IN *TRYPANOSOMA GAMBIENSE* TREATED WITH VINBLASTINE, COLCHICINE PLUS CONCANAVALIN A.

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The effects of vinblastine alone and in combination with vinblastine, colchicine and concanavalin A on microtubules of *Trypanosoma gambiense* cultured in vitro were studied ultrastructurally. Trypanosomes treated with vinblastine at 20 μg/ml, showed fusion of the extracellular flagellum with the plasma membrane of the parasite. As a result, the axoneme with the paraxial rod in the extracellular flagellum was taken into the cytoplasm. Although the axonemal and pellicular microtubules in *T. gambiense* differ in function and origin, the axonemal microtubules of the extracellular flagellum that was taken into the cytoplasm could be converted to pellicular microtubules by treatment with a combination of vinblastine (20 μg/ml), concanavalin A (10 μg/ml) and colchicine (100 μg/ml).

Ono and Nakabayashi (1979) found that treatment of *Trypanosoma gambiense* with vinblastine and colchicine caused disorder in the arrangement of axonemal microtubules of the extracellular flagellum and increased the formation of both protofilaments and axonemes. Moreover, Hoffstein et al. (1976) reported that concanavalin A induced the assembly of microtubules in the cytoplasm of leucocytes. In the present experiment, therefore, the effects of vinblastine alone and in combination with colchicine and concanavalin A on the microtubules of *Trypanosoma gambiense* were studied ultrastructurally. The result showed that in trypanosomes treated with vinblastine, colchicine plus concanavalin A, microtubules in the axoneme of the extracellular flagellum, which is taken into the cytoplasm, are converted to pellicular microtubules.

The Wellcome strain of *Trypanosoma gambiense* was used. This strain has been maintained in this laboratory by serial passage in mice. However, we used parasites cultured

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in vitro in this experiment. When trypanosomes had reached a level of 8×10^8/ml in the blood stream of Swiss albino mice after inoculation of approximately 5×10^4 trypanosomes, the blood was collected in 0.3% sodium citrate-0.85% saline solution. A sample of 1.5 ml of blood containing 1.5×10^7 trypanosomes /ml was suspended in a mixture of 12 ml of Eagle's minimal essential medium and 1.5 ml of calf serum, and trypanosomes were cultivated with 20 μg/ml of vinblastine (Kyorin Seiyaku) and 10 μg/ml of concanavalin A (Sigma) at 37 C for 2 h. Colchicine (Wako Pure Chemical) was also added at a final concentration of 100 μg/ml and 19 h later the trypanosomes were collected by centrifugation. In some experiments, trypanosomes were cultivated with 20 μg/ml of vinblastine alone at 37 C for 19 h. The samples were washed with 0.85% saline solution and fixed with 2.5% glutaraldehyde in 0.01 *M* phosphate buffer (pH 7.4) at 4 C for 1 h. The parasites were washed four times with 0.01 *M* phosphate buffer containing 0.25 *M* sucrose and post-fixed in 1.5% osmium tetraoxide for 1 h at room temperature. The samples were stained with a saturated solution of uranyl acetate in 50% ethanol for 1 h. After dehydration with ethanol, the samples were embedded in low viscosity epoxy resin by the method of Spurr (1969). Sections were cut and stained with uranyl acetate and lead citrate.

An electron micrograph of *T. gambiense* before drug treatment is shown in Fig. 1A. An axoneme and paraxial rod are seen in the extracellular flagellum. The axoneme is composed of microtubules (arrow), nine peripheral doublets and two central microtubules. Pellicular microtubules (arrow head) are visible in a single layer below the plasma membrane of the parasite. Figure 1B shows a trypanosome treated with 20 μg/ml of vinblastine for 19 h in vitro. Fusion of the extracellular flagellum with the plasma membrane of the parasite is seen. Consequently, the axoneme with the paraxial rod of the extracellular flagellum is taken into the cytoplasm. Trypansomes that had been exposed to vinblastine (20 μg/ml) and concanavalin A (10 μg/ml), were cultivated for 19 h after addition of colchicine at a final concentration 100 μg/ml (Fig. 1C, D). In Fig. 1C, an axoneme with paraxial rod is seen in the cytoplasm. No pellicular microtubules are observed below the plasma membrane of the parasite surrounding this axoneme. Findings were similar in trypanosomes treated with vinblastine alone at 20 μg/ml for 19 h. The axoneme with the paraxial rod shown in Fig. 1C appears to be incorporated into the cytoplasm due to fusion of the extracellular flagellum with the parasite plasma membrane, as shown in Fig. 1B. The direction of pellicular microtubules in a protruding part of the cytoplasm is different from that of pellicular microtubules in the main part of the cytoplasm. But, it is unclear whether fusion of the extracellular flagellum with the plasma membrane of the parasite is related with formation of the protruding part of the cytoplasm.

In the axoneme with a paraxial rod shown in Fig. 1D, the peripheral doublets #5 and #6, numbered according to Afzelius' nomenclature (1959), are not observed. On the other hand, microtubules of two doublets (double arrow) are observed below the parasite plasma membrane rather far from the usual location of peripheral doublet microtubules of an axoneme. We did not examine further how these doublets of axonemal microtubules developed, but the present findings suggest conversion of axonemal microtubules to pellicular microtubules.

There are three types of microtubules in *T. gambiense*, pellicular, axonemal and nuclear spindle types, which differ in function and origin. In the present, ultrastructural findings suggest that axonemal microtubules can be converted to pellicular microtubules in specific conditions on treatment with vinblastine, colchicine plus concanavalin A in vitro. However, it is unknown whether all these drugs are required for conversion of axonemal microtubules or how the actions of these drugs result in the conversion of axonemal micro-

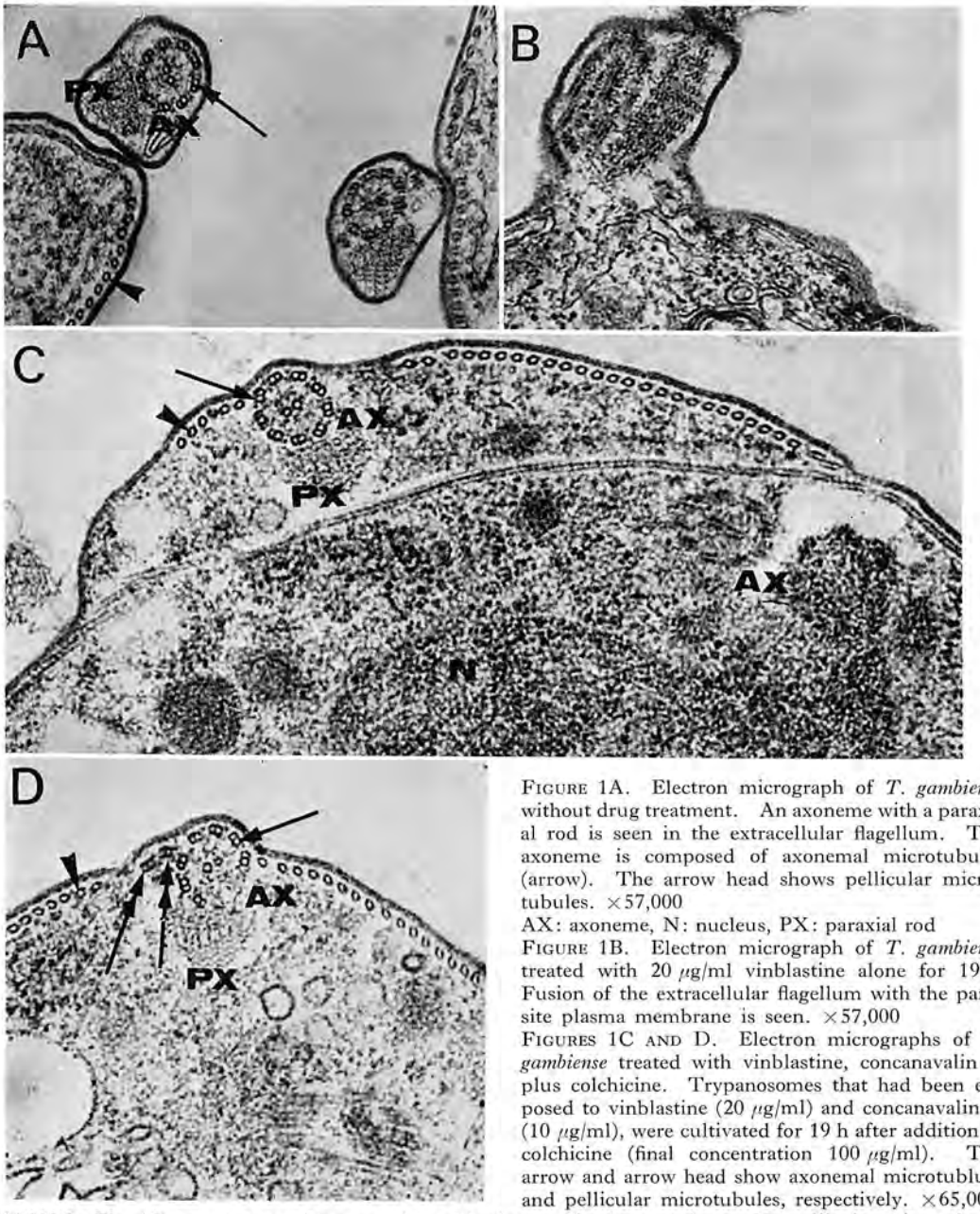

FIGURE 1A. Electron micrograph of *T. gambiense* without drug treatment. An axoneme with a paraxial rod is seen in the extracellular flagellum. The axoneme is composed of axonemal microtubules (arrow). The arrow head shows pellicular microtubules. ×57,000
AX: axoneme, N: nucleus, PX: paraxial rod
FIGURE 1B. Electron micrograph of *T. gambiense* treated with 20 μg/ml vinblastine alone for 19 h. Fusion of the extracellular flagellum with the parasite plasma membrane is seen. ×57,000
FIGURES 1C AND D. Electron micrographs of *T. gambiense* treated with vinblastine, concanavalin A plus colchicine. Trypanosomes that had been exposed to vinblastine (20 μg/ml) and concanavalin A (10 μg/ml), were cultivated for 19 h after addition of colchicine (final concentration 100 μg/ml). The arrow and arrow head show axonemal microtublues and pellicular microtubules, respectively. ×65,000, ×65,000 In Fig. 1C, an axoneme with a paraxial rod is observed in the cytoplasm. No pellicular microtubules are observed below the plasma membrane surrounding the axoneme in the parasite. This axoneme appears to be included in the cytoplasm due to fusion of the extracellular flagellum with the plasma membrane of the parasite, as shown in Fig. 1B. In Fig. 1D, no peripheral doublets #5 and #6, numbered according to Afzelius' nomenclature, are observed in the axoneme. On the other hand, microtubules of two doublets (double arrow) are observed below the plasma membrane of the parasite rather far from the usual location of peripheral doublet microtubules of an axoneme.

tubules to pellicular microtubules. The antitubulin drugs vinblastine and colchicine, and the mitogenic substance concanavalin A, have effects on not only microtubules of the cells but also the function of the cytoplasmic membrane. Therefore, the ultrastructural changes observed in this study may have been brought about through effects on both microtubules and the cytoplasmic membrane.

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