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Studies on Effect of Concanavalin A and Cytochalasin B on *Trypanosoma gambiense*

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Abstract

Effects of two drugs, concanavalin A and cytochalasin B, on *Trypanosoma gambiense* were examined morphologically. Concanavalin A caused excess assembly of pellicular microtubules and disorderly arrangement of the axonemal and pellicular microtubules. But, this drug did not induce the anucleate form. On the other hand, in treatment with cytochalasin B known to have effect on microfilament, neither abnormality of microtubules nor induction of anucleate form were observed. Cytochalasin B caused delay or inhibition of cytoplasmic division without inhibitory effect on nuclear division and consequently induced the formation of multinucleate parasites. Moreover, various effects of cytochalasin B in the treated-trypanosomes were observed. Concanavalin A and cytochalasin B were found to have no ability to induce dyskinetoplastic form.

Key words: Concanavalin A, Cytochalasin B, *Trypanosoma gambiense*

Introduction

In a series of ultrastructural studies on the effects of various drugs on trypanosomes, we reported that neocarzinostatin, vinblastine, colchicine and bleomycin resulted in disorganization of microtubules and induction of the anucleate form (Ono and Nakabayashi, 1978, 1979, 1980). However, we never observed any abnormality of microtubules in trypanosomes treated with chemicals inducing the dyskinetoplastic form (Ono and Inoki, 1971, 1973, 1974, 1975, 1976).

Therefore, to examine in detail the relation between disorganization of microtubules and induction of anucleate form of *T. gambiense*, effects of two drugs, concanavalin A and cytochalasin B, on *T. gambiense* were examined in the present experiment. Concanavalin A is known to have effect on microtubules, but this drug is not known to inhibit nucleic acid synthesis unlike anucleate form-inducing chemicals, neocarzinostatin, bleomycin, colchicine and vinblastine used in the previous reports (Ono and Nakabayashi, 1978, 1979, 1980). On the other hand, cytochalasin B has no effect on microtubules, but has effect on microfilament. The result indicated that neither anucleate form nor dyskinetoplastic form were induced in *T. gambiense* treated with concanavalin A and cytochalasin B.

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Materials and Methods

1. *Species of Trypanosoma*

The Wellcome strain of *Trypanosoma gambiense* was used. This strain was the same as that used in our previous report (Ono and Nakabayashi, 1980). This strain has been maintained in this laboratory by serial passages in mice for more than 20 years. All the trypanosomes of this strain had either one nucleus or two nuclei. The percentages of dyskinetoplastic forms are less than 1%.

2. *Light and electron microscopic observations*

Experimental methods were substantially the same as described in our previous report (Ono and Nakabayashi, 1979). When trypanosomes had reached a level of 8×10^8 trypanosomes/ml in the blood stream in Swiss albino mice 3 days after intraperitoneal inoculation with 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 treated with drug as follows.: (1) Incubated with 20 μg/ml concanavalin A (Sigma) for 24 hr at 37°C, and used for electron microscopic observation. (2) Incubated with 5 μg/ml cytochalasin B (Sigma) or 20 μg/ml concanavalin A for 5 hr, and injected intraperitoneally into Swiss albino mice and 5 or 21 hr later, trypanosomes were collected from the peritoneal fluid or peripheral blood of the mice. And then, trypanosomes were examined by light and electron microscopy. Electron microscopy was carried out as described by Ono and Inoki (1976), except that materials were embedded in low viscosity epoxy resin by the method of Spurr (1969).

Results

Figure 1A shows a light micrograph of *T. gambiense* not treated with drug. Division of

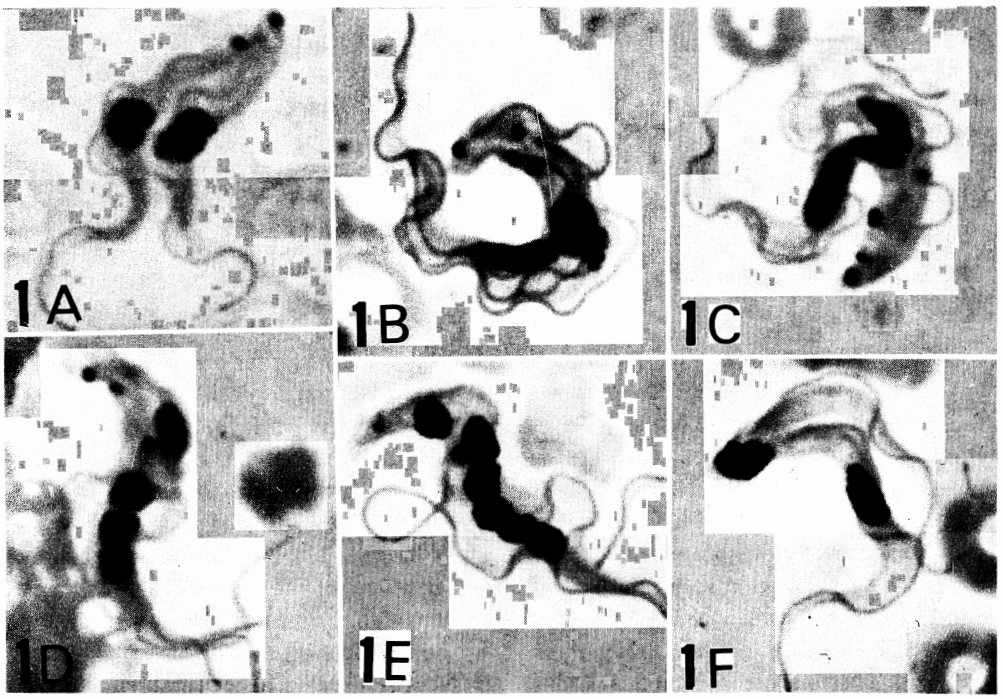

Fig. 1A Light micrograph showing *T. gambiense* untreated with drug. ×2,400.

Figs. 1B–1F Light micrographs showing cytomorphological changes in *T. gambiense* obtained from peritoneal fluid of mice 5 hr after intraperitoneal injection of trypanosomes previously incubated with 5 μg/ml cytochalasin B for 5 hr. ×2,700.

In Fig. 1B, a trypanosome which has four kinetoplasts and two nuclei, dose not show any sign of cytoplasmic division. In a dividing form in Fig. 1C, cleavage furrow in cytoplasm appears to be stopped at site of a horseshoe-shaped nucleus. Figs. 1D and E show aberrant multinucleate trypanosomes. But, cell size is almost same as untreated control in Fig. 1A. Fig. 1F shows a dividing form in which a nucleus removes to the usual location of kinetoplast.

trypanosomes begins in the basal body. And then, it is followed by binary fission of the kinetoplast and later by division of the nucleus and cytoplasm. As in Fig. 1A, therefore, when two kinetoplasts separate moderately, two daughter trypanosomes are already produced.

Figure 1B-1F show light microscopically the cytomorphological changes in trypanosomes obtained from peritoneal fluid of mice 5 hr after intraperitoneal injection of trypanosomes previously incubated with 5 μg/ml cytochalasin B for 5 hr.

In Fig. 1B, although a trypanosome has four kinetoplasts and two nuclei, it does not show any sign of cytoplasmic division. In Fig. 1C, a trypanosome has already four kinetoplasts and two large nuclei, but its cleavage furrow in cytoplasm appears to be stopped at site of a horseshoe-shaped nucleus. Figures 1D and E show abnormal multinucleate trypanosomes. But, cell volume is similar size in comparison with untreated control in Fig. 1A. Figure 1D shows a trypanosome in which three kinetoplasts and four nuclei are seen. In Fig. 1E, although one kinetoplast was observed in a trypanosome with six nuclei, a large majority of dividing forms with more than two nuclei in trypanosomes treated with cytochalasin B have usually two or four kinetoplasts. Figure 1F shows a dividing form with two nuclei. A nucleus moves to the usual location of kinetoplast. Any kinetoplast can not be seen in the cytoplasm. In trypanosomes treated with cytochalasin B, morphological alterations were observed in approximately 5% of total parasites.

Trypanosomes treated with concanavalin A did not show any light microscopically recognizable alteration. Neither anucleate form nor dyskinetoplastic forms are detectable after treatment with cytochalasin B or concanavalin A.

Figure 2 shows an electron micrograph of untreated *T. gambiense*. The nucleus is elliptical and the nucleolus is situated slightly eccentrically as a large electron-dense finely granular spherical structure. Pellicular microtubules are seen in a single layer below the plasma membrane. Figure 3 shows trypanosome incubated with 20 μg/ml concanavalin A for 24 hr in vitro. A large number of microtubules are seen in the cytoplasm far from and below the plasma membrane.

Figures 4 and 5 show trypanosomes obtained from mice 21 and 5 hr after intraperitoneal injection of trypanosomes previously incubated with 20 μg/ml concanavalin A for 5 hr in vitro. In Fig. 4, a bud-like protrusion in which density of the cytoplasm was remarkably high, is seen. There are no pellicular microtubules below the plasma membrane of a bud-like protrusion. Disappearance of pellicular microtubules is observed in the vicinity of the base of a bud-like protrusion. Disordered arrangement of axonemal microtubules of the extracellular flagellum was observed (Fig. 5). We could not observe ultrastructurally any recognizable alteration in the nucleus and the kinetoplast.

Figures 6–9 show trypanosomes obtained from mice 5 hr after intraperitoneal injection of trypanosomes previously incubated with 5 μg/ml cytochalasin B for 5 hr in vitro. In Fig. 6, the division of the cytoplasm proceeds by incision, but it appears to be stopped at site of a incompletely divided nucleus.

In Fig. 7, two parasites are connected by small part of the cytoplasm. In Fig. 8, a flagellum with paraxial rod is seen projecting from the cytoplasm at one end of trypanosome. Neither flagellar pocket nor maculae adherentes

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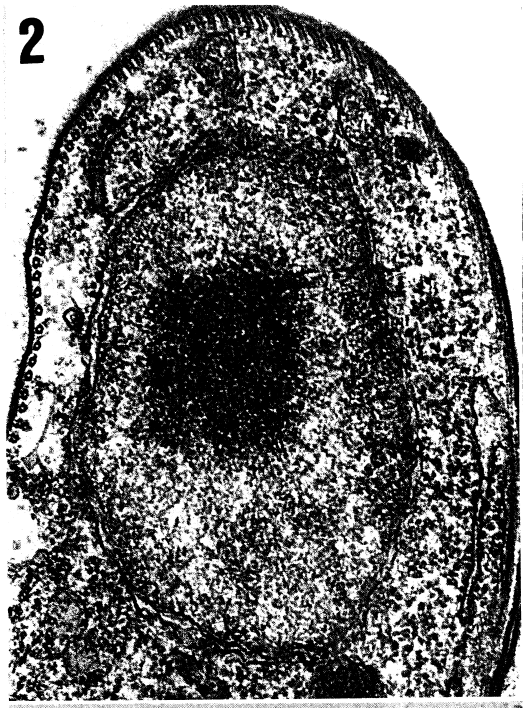
Fig. 2 An electron micrograph showing *T. gambiense* untreated with drug. ×52,500. Pellicular microtubules are seen in a single layer below the plasma membrane.

Fig. 3 shows trypanosome incubated with 20 μg/ml concanavalin A for 24 hr in vitro. ×75,000. A large number of microtubules are seen in the cytoplasm far from and below the plasma membrane.

Fig. 4 and 5 show trypanosomes obtained from mice 21 and 5 hr after intraperitoneal injection of trypanosomes previously incubated with 20 μg/ml concanavalin A for 5 hr in vitro. ×44,000, ×60,000.
In Fig. 4, a bud-like protrusion in which density of the cytoplasm remarkably high, is seen. Disordered arrangement of axonemal microtubules of the extracellular flagellum was observed (Fig. 5).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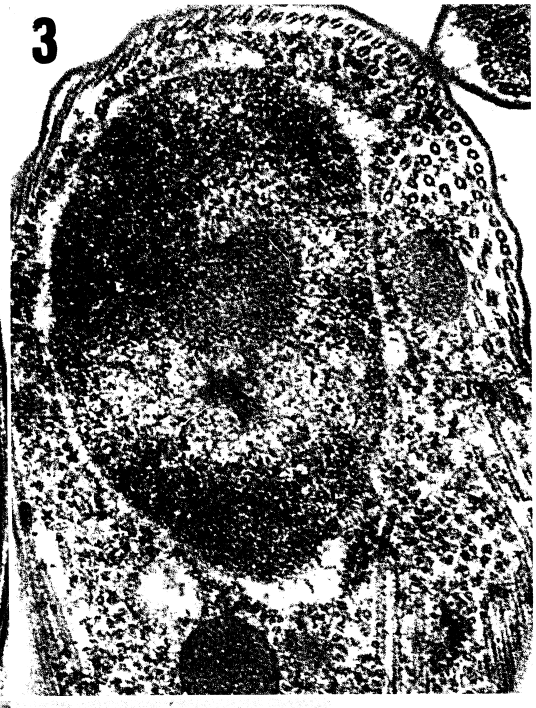
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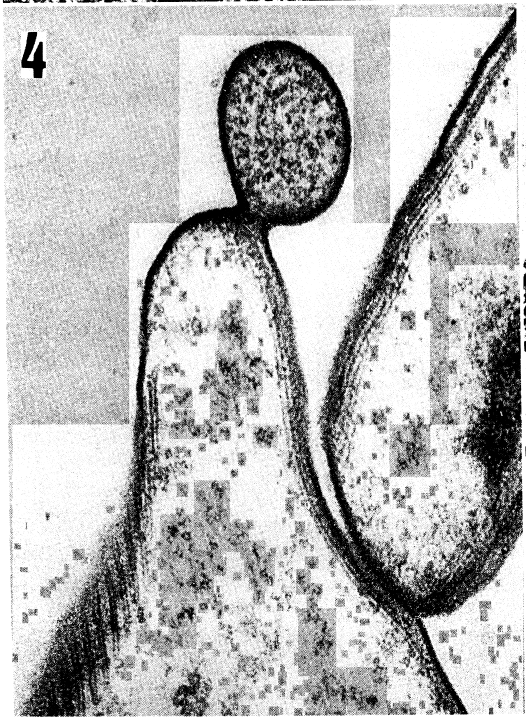
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are observed at the base of the flagellum. In Fig. 9, membrane-bounded four flagella in various sectional planes are seen in the cytoplasm. The paraxial-rod which is observed only in the extracellular flagellum in untreated trypanosomes, was found in three intracellular flagella.

Discussion

In the previous reports, we found that anucleate form-inducing substances, neocarzinostatin, bleomycin, colchicine and vinblastine which cause depression of DNA synthesis or inhibition of DNA dependent RNA synthesis, resulted in excess assembly of pellicular microtubules and disorderly arrangement of the axonemal and pellicular microtubules (Ono and Nakabayashi, 1978, 1979, 1980). Whereas, dyskinetoplast form-inducing substances, pararosaniline, acriflavine, furazolidon, ethidium bromide, hydroxy-stilbamidine which are known to interact with DNA, did not result in disorganization of microtubules (Ono and Inoki, 1971, 1973, 1974, 1975). In the present experiment, therefore, effect of concanavalin A and cytochalasin B on *T. gambiense* was observed. Concanavalin A and cytochalasin B have ability to interact with microtubules and microfilaments, respectively, and both chemicals do not inhibit nucleic acid synthesis.

The result indicated neither anucleate form nor dyskinetoplastic form was observed on treatment with concanavalin A and cytochalasin B. This fact may suggest that anucleate forms are induced by drugs having both of inhibitory effect on nucleic acid synthesis and effect on microtubules.

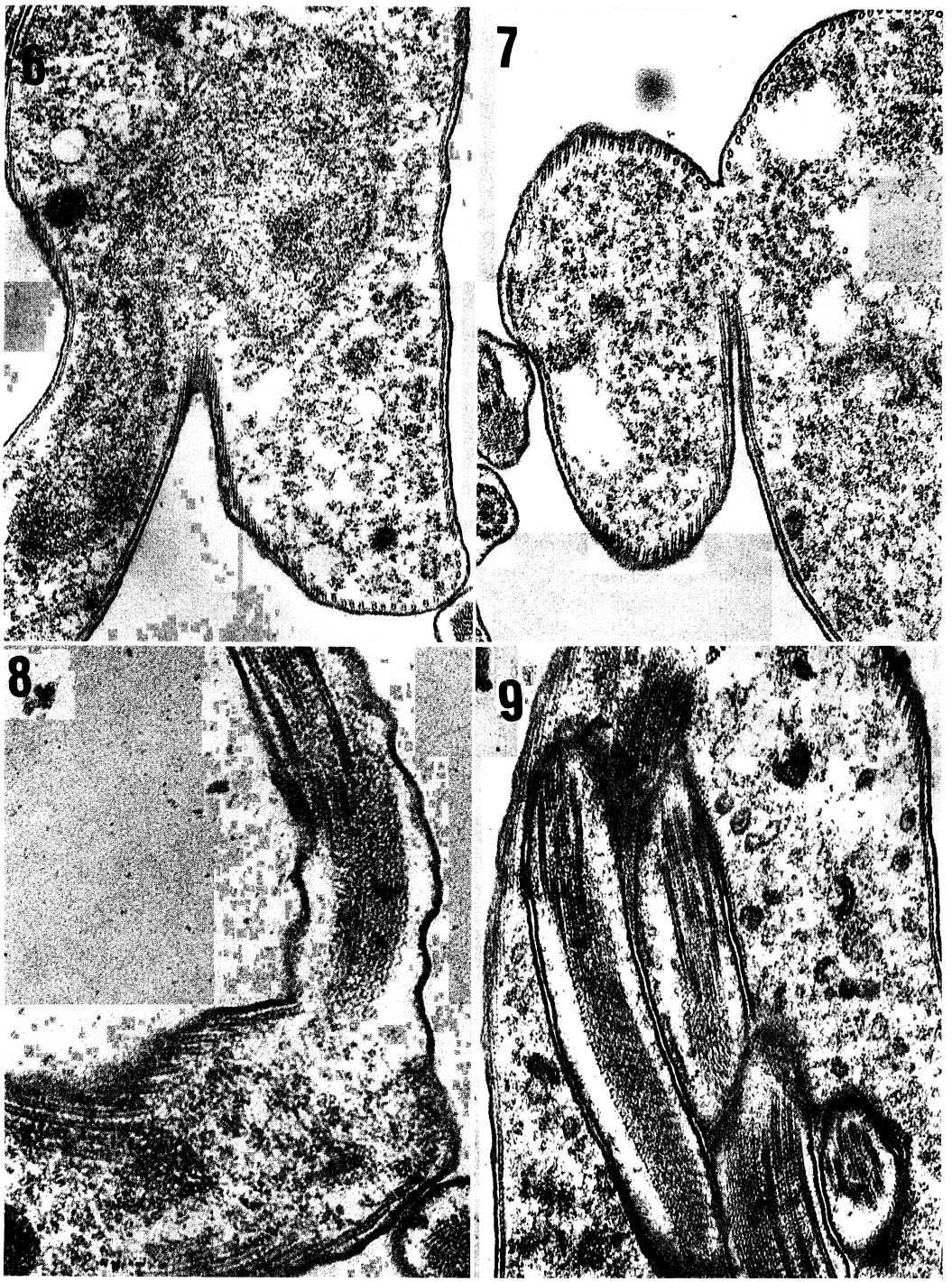
Hoffstein *et al.* (1976) reported that in human leucocytes treated with concanavalin A (100 μg/ml) remarkably enhanced numbers of microtubules appeared in the cytoplasm. We also observed a large number of microtubules in the cytoplasm far from and below the plasma membrane in *T. gambiense* treated with 20 μg/ml of concanavalin A (Fig. 3).

In previous report, treatment of *T. gambiense* with colchicine resulted in formation of cytoplasmic projections that appeared as protrusions from a small part of the surface membrane (Ono and Nakabayashi, 1979). Similar finding was observed in trypanosomes treated with concanavalin A in the present experiment (Fig. 4). Disorder in arrangement of pellicular microtubules which was observed on treatment with all chemicals capable of inducing the anucleate form (Ono and Nakabayashi, 1978, 1979, 1980), was also induced in trypanosomes treated with concanavalin A (Fig. 5).

Carter (1967) and Krishan (1971) reported that in mouse fibroblast cells treated with cytochalasin B, normal nuclear divisions were observed, but cytoplasmic cleavage furrow failed to separate the cells completely, and the cell reunited to form binucleate cells. Repetition of this process resulted in the formation of large multinucleate cells. In the present experiment, however, cell size of dividing form with four or six nuclei is almost the same in comparison with untreated control (Figs. 1A, D, E). Furthermore, many multinucleate parasites did not show any sign of cytoplasmic division (Figs. 1B, D, E). Thus, the above findings seem to indicate that multinucleate forms of trypanosomes appearing at that time when treated with cytochalasin B are produced as a result of delay or inhibition of cytoplasmic division.

In tryapnosomes treated with cytochalasin B, cytoplasmic division ceased in the neighbour-

Fig. 6–9 show trypanosomes obtained from mice 5 hr after intraperitoneal injection of trypanosomes previously incubated with 5 μg/ml cytochalasin B for 5 hr in vitro.
In Fig. 6, the division of the cytoplasm proceeds by incision, but it appears to be stopped at site of an incompletely divided nucleus. ×32,000. Fig. 7 shows two parasites connected by small part of the cytoplasm. ×42,000. In Fig. 8, a flagellum with paraxial rod is seen projecting from the cytoplasm at one end of trypanosome. ×60,000. Neither flagellar pocket nor maculae adherentes are observed at the base of the flagellum. In Fig. 9, four flagella were found in the cytoplasm. ×42,000. The paraxial rod which is observed only in the extracellular flagellum in untreated trypanosomes, was observed by the side of axoneme in three flagella.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hood of a horseshoe-shaped nucleus (Figs. 1C and 6) and only one kinetoplast is observed in a trypanosome with six nuclei (Fig. 1E). These findings show seemingly delay or inhibition of nuclear and kinetoplast division. But, dividing forms with more than two nuclei have usually more than two kinetoplasts, as in Figs. 1B, C, D. Cytochalasin B is found to have no ability to inhibit nuclear and kinetoplast division. Disorder in continuity and regularity of nuclear and kinetoplast division due to inhibition or delay of cytoplasmic division may resulted in appearance of such aberrant dividing forms in a part of trypanosomes treated with cytochalasin B (Figs. 1C, E and 6).

Carter (1967) observed extrusion of nucleus in mouse fibroblast cells treated with 1 – 10 μg/ml cytochalasin B. In the present experiment, a nucleus located in the central area of cytoplasm moved to the posterior end of trypanosome treated with 5 μg/ml cytochalasin B (Fig. 1F). But, extrusion of nucleus was not observed. Existence of pellicular microtubules in trypanosomes may prevent extrusion of nucleus from cytoplasm.

The paraxial rod which is observed only in the extracellular flagellum in untreated trypanosomes, was found by the side of axoneme in the cytoplasm of a cytochalasin B-treated trypanosome (Fig. 9). Moreover, projection of a flagellum from cytoplasm without flagellar pocket and maculae adherentes was observed. These findings indicate various effects of cytochalasin B on trypanosomes.

References

1) Carter, S. B. (1967): Effects of cytochalasins on mammalian cells. Nature, 213, 261–264.
2) Hoffstein, S., Soberman, R., Goldstein, I. and Weissmann, G. (1976): Concanavalin A induced microtubules assembly and specific granule discharge in human polymorphonuclear leucocytes. J. Cell Biol., 68, 781–787.
3) Krishan, A. (1971): Fine structure of cytochalasin-induced multinucleated cells. J. Ultrastruct. Res., 36, 191–204.
4) Ono, T. and Inoki, S. (1971): Studies on the effect of p-rosaniline resistant *Trypanosome evansi*. Biken J., 14, 419–422.
5) Ono, T. and Inoki, S. (1973): Chemotherapy and akinetoplastic form induction test with furazolidon in *Trypanosoma gambiense* infected mice. Jpn. J. Parasitol., 22, 320–330.
6) Ono, T. and Inoki, S. (1974): Effects of AK-form inducing substances on mitomycin-C treated *Trypanosoma gambiense* and *Trypanosoma evansi* in mice. Jpn. J. Parasitol., 23, 58–65.
7) Ono, T. and Inoki, S. (1975): Effect of hydroxystilbamidine on the kinetoplast of *Trypanosoma gambiense* in mice. Biken J., 18, 257–265.
8) Ono, T. and Inoki, S. (1976): Electron microscopic observations on the effect of human serum, anti-trypanosome mouse-serum and acriflavine on *Trypanosoma gambiense* in mice. Jpn. J. Parasitol., 25, 47–58.
9) Ono, T. and Nakabayashi, T. (1978): Effect of neocarzinostatin on the nucleus, kinetoplast and microtubules of *Trypanosoma gambiense* and *Trypanosoma evansi*. Biken J., 21, 161–172.
10) Ono, T. and Nakabayashi, T. (1979): Studies on the effects of vinblastine and colchicine on *Trypanosoma gambiense*. Biken J., 22, 117–124.
11) Ono, T. and Nakabayashi, T. (1980): Studies on the effect of bleomycin on *Trypanosoma gambiense*. Biken J., 23, 143–155.
12) Spurr, A. R. (1969): A low viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastruct. Res., 26, 31–43.