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ELECTRONMICROSCOPIC OBSERVATIONS ON LYSIS OF *TRYPANOSOMA CRUZI* EPIMASTIGOTE BY NORMAL RABBIT SERUM

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SUMMARY The lytic effects of serum from a non-immunized rabbit on epimastigotes of *Trypanosome cruzi* were studied by electronmicroscopy. The first detectable change was the appearance of a fuzzy deposit over the whole surface of the epimastigote. Soon after this, pellicular microtubules disappeared without change of axonemal microtubules. Circular lesions were observed by negative staining, corresponding to the lesion of antibody-mediated lysis caused by complement.

INTRODUCTION

Fresh sera from various species of nonimmunized animals can lyse the epimastigote form of *Trypanosoma cruzi*, but not the trypomastigote or amastigote form. Therefore we investigated the surface characters of these three forms by electronmicroscopy and immunofluorescence, and found a coating substance on the membranes of the trypomastigote and amastigote forms that was not present on membranes of the epimastigote form (Kanbara et al., 1974; Kanbara, 1975).

The lytic effect of serum is destroyed by heating it at 56 C for 30 min, and the effect is restored by addition of fresh mouse serum that originally has no lytic effect (Rubio, 1956). Warren (1958) examined the mechanism of the lytic action of normal chicken serum and reported that fowl serum contains at least two active factors, probably a heat-stable antibody and a heat-labile complement.

Anziono et al. (1972) studied the immune lysis of culture forms of *Trypanosoma cruzi* using sera from immunized rabbits, infected and normal humans, and normal guinea pigs and showed that antibody-mediated lysis is caused by complement, which is activated via the classical pathway. Recently Nogueira (1975) reported that lysis of the epimastigote form by human, rabbit and guinea pig sera was dependent upon the alternate pathway of complement activation.

In this study, we observed the course of the lytic reaction of normal rabbit serum on epimastigotes by electronmicroscopy.

MATERIAL AMD METHODS

1. *Trypanosomes*

The Tulahuen strain of *T. cruzi* was obtained from the National Institute of Health, Bethesda, Maryland, U.S.A. through the Parasitological Department of Keio University, Tokyo, Japan. Cultures in monophasic medium in which 98 percent of the parasites were epimastigotes were used. The monophasic medium consisted of 94% base-solution (1.0% trypticase, 0.8% NaCl and 0.5% glucose in distilled water), 5% calf-serum and 1% sheep red cell extract. For preparation of the red cell extract, sheep red cells were washed twice with saline with centrifugation at 3,000 rpm for 10 min, and hemolysed by adding the same volume of distilled water as that of the original blood. Then the preparation was centrifuged at 10,000 rpm for 30 min and the supernatant was sterilized by filtration and used as sheep red extract.

2. *Normal serum*

Serum from adult nonimmunized rabbits was used in studies on lysis of epimastgotes.

3. *Electronmicroscopy*

Two milliliters of washed, precipitated trypanosomes from culture were suspended in 10 ml of saline and divided into 1 ml samples.

Each sample was mixed with 2 ml of rabbit serum. After incubation for 1, 2, 3, 5, 10, 30, and 60 min at room temperature, samples were mixed with 40 ml of phosphate buffer (0.1 M, pH 7.4) containing 2.5% glutaraldehyde and kept at 4 C for 40 min for fixation. Then the samples were washed at least 3 times with phosphate buffer and post-fixed in 1.0% osmium tetroxide in phosphate buffer at 4 C for 1 hr. The specimens were dehydrated in a series of increasing concentrations of ethanol and then transferred to glycidyl n-buthyl ether and embedded in Epon mixture. Sections were stained first with uranyl ace-

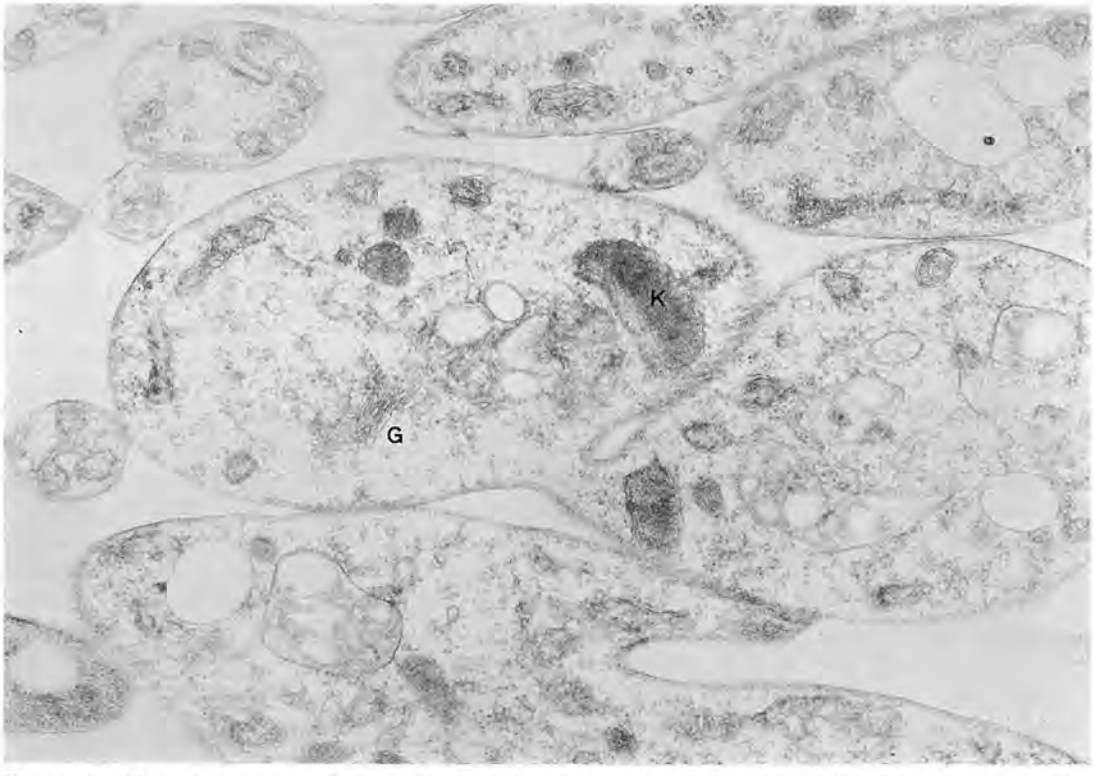


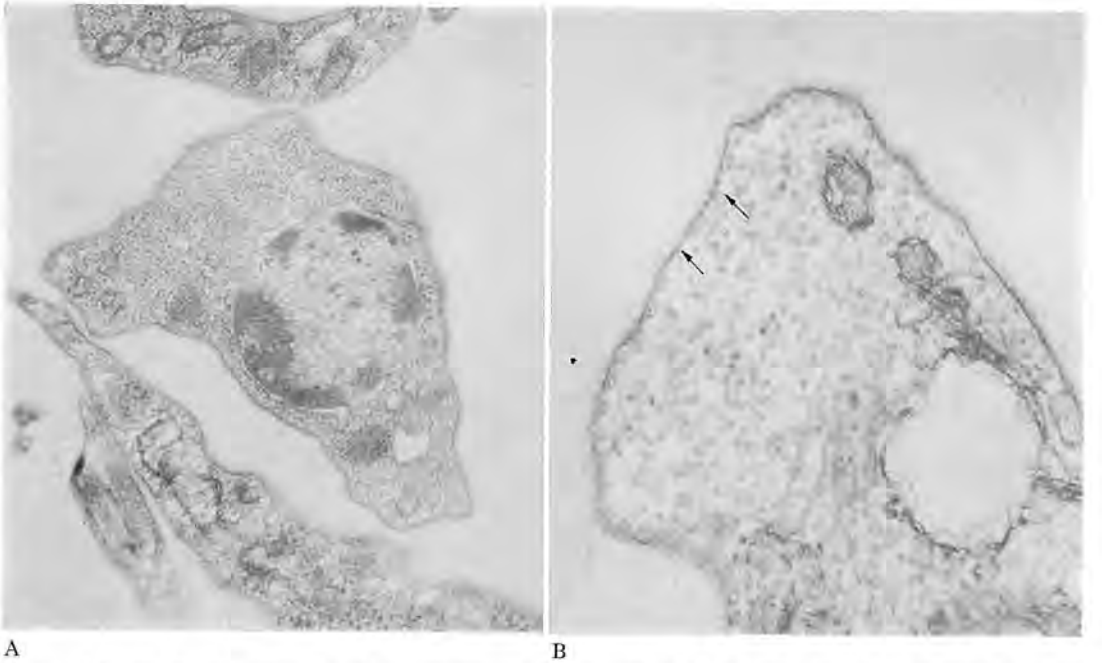
FIGURE 1. Normal structure of epimastigote before serum treatment. (×34,300) K: Kinetoplast, G: Golgi-apparatus.

tate for 30 min at 37 C and then with lead citrate for 10 min at room temperature.

The remainder of the samples that had been incubated with rabbit serum for 60 min were washed with saline by centrifugation at 3,000 rpm for 20 min. The precipitates were next washed twice with 0.01 M phosphate buffer (pH 7.0) by high-speed centrifugation at 10,000 rpm and 4 C for 1 hr and the pellets were suspended in phosphate buffer. Drops of the suspension were placed on collodion coated grids and negatively stained with 2% aqueous uranyl acetate. Micrographs were taken with a Hitachi 11B electron-microscope.

RESULTS

No visible change was observed under an electromicroscope after 2 min incubation. After 3 min, the surface of the parasites became diffusely coated with fuzzy material and some pellicular microtubules disappeared (Fig. 2A, B). After 4 min, all pellicular microtubules had disappeared, but axonemal microtubules were not affected (Fig. 3A, B, C). The cell surface became rough and had projections from which material seemed to ooze out (Fig. 3C). The membrane of the nucleus as well as the cisternae of the Golgi-apparatus and the endoplasmic reticulum (ER) became partially swollen, while the membranes of the mitochondria and the kinetoplast showed little change (Fig. 3B). The cytoplasm gradually lost its density with time. After 5 min, projections from the plasma-membrane increased in number and size, and some of them developed into small vesicles which were structurally similar to the plasma membrane. After 10 min, the damage of the membranes of the nucleus, the Golgi-apparatus and the ER had increased. At this time, the mitochondria were also affected, their cristae becoming swollen and rounded. After 30 min, the plasma-membrane had lost its projections and become smooth and round, and breaks in it were seen here and there under high magnification. The kinetoplast and the flagellar structure showed



A B

FIGURE 2A, and B. Three min after serum treatment, the surface of the plasma membrane becomes covered with a fuzzy substance and some microtubules disappear (arrows). (Fig. 2A ×30,000, Fig. 2B ×90,000)

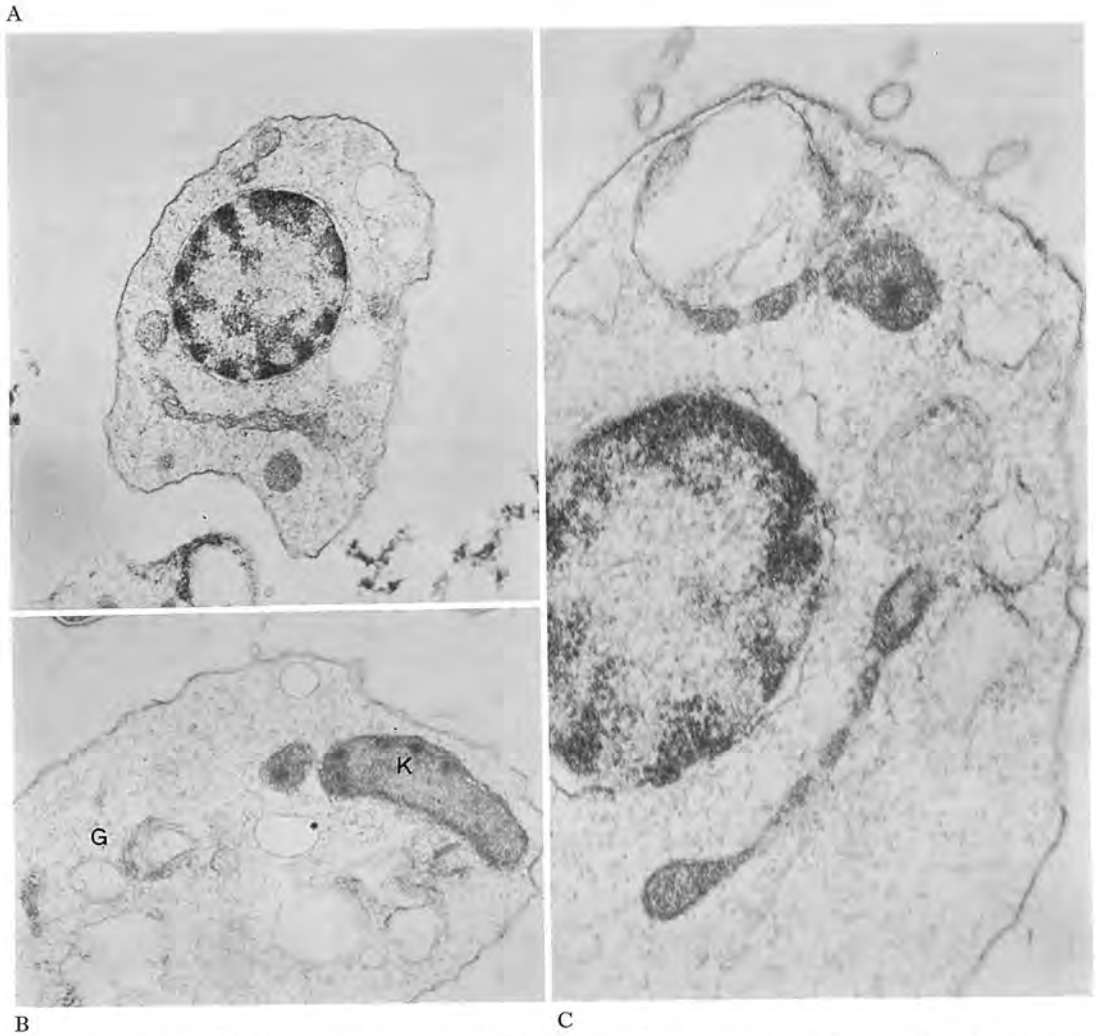
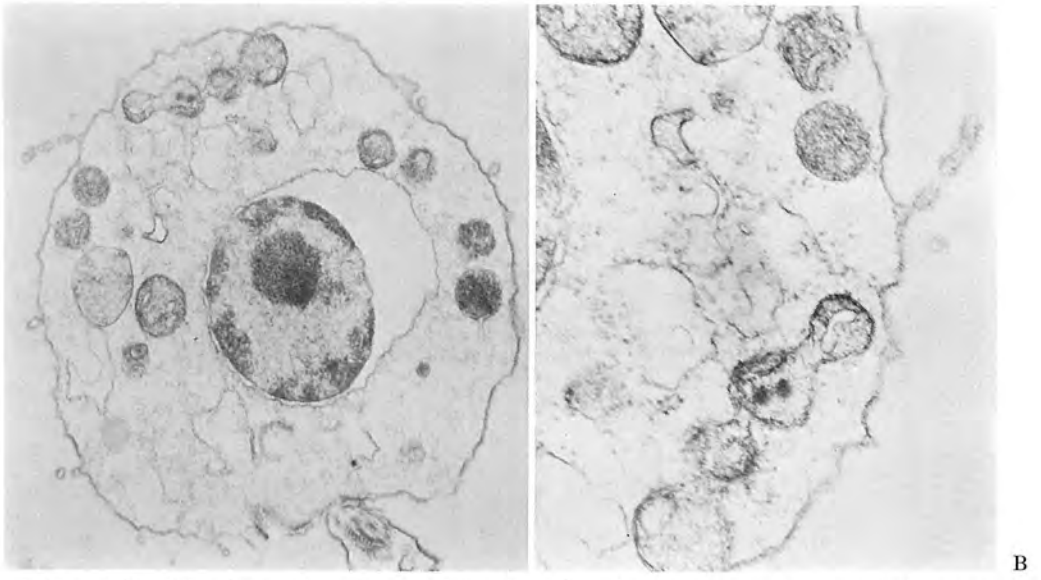


FIGURE 3A, B and C. After 4 min, all pericular microtubules have disappeared. The cell surface becomes rough and has projections. The nuclear membrane and Golgi-apparatus become slightly swollen. The density of the cytoplasm decreases slightly. (Fig. 3A ×30,000, Fig. 3B ×45,000, Fig. 3C ×70,000)

little change, but the nucleus became swollen, and the chromatin became denaturated and other organellae, such as the ER and the Golgi-apparatus, disappeared (Fig. 5A, B). After 60 min, changes were similar to those after 30 min, but more extensive (Fig. 6). Negative staining showed regular circular lesions with outer and inner diameters of about 75 Å and 60 Å, respectively, in the membrane (Fig. 7).

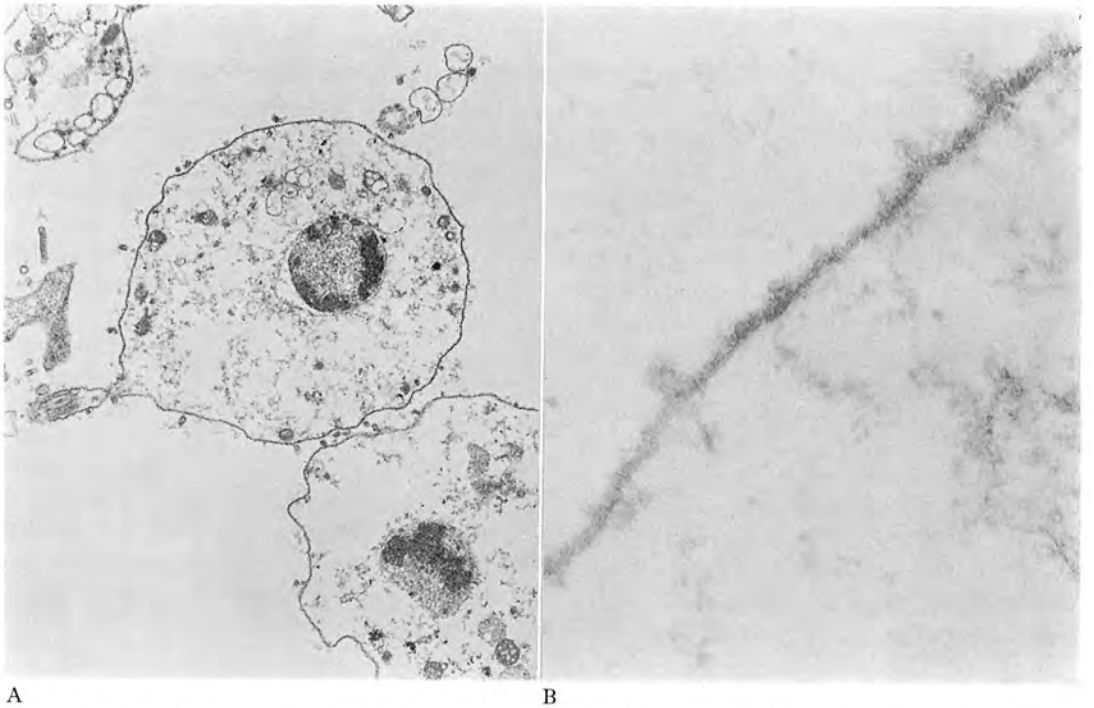
DISCUSSION

Since the report of Minz and Boriello (1945), the lytic effects of normal sera from various animals on the epimastigote form of *T. cruzi* have been studied extensively, and it has been suggested that the observed changes involve immune lysis, which requires complement. Rubio (1956) noted that inactivated serum



A B

FIGURE 4A and B. After 5 min, the projection from the cell surface increase in size and number and some of them develop into vesicles. (Fig. 4A ×20,000, Fig. 4B ×60,000)



A B

FIGURE 5A and B. After 30 min, the plasma membrane becomes smooth and round. It shows breaks here and there and is coated with adherent substances. The cytoplasm has become almost transparent. (Fig. 5A ×15,000, Fig. 5B ×149,040)

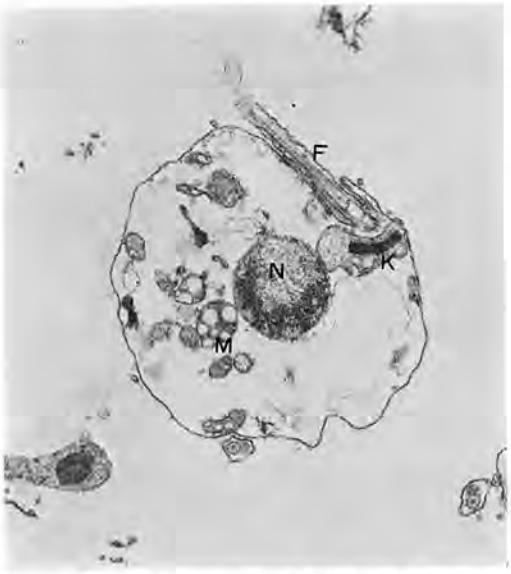


FIGURE 6. After 60 min, only the nucleus, mitochondria including the kinetoplast and flagellum remain intact. (×15,000)
N: nucleus, M: mitochondria, F: flagellum

FIGURE 7. Circular lesions in the plasma membrane shown by negative staining. (×180,000)

which has lost its lytic effect regained its lytic effect on addition of fresh mouse serum, which is known to have no lytic activity and a very low complement titer.

In the present electronmicroscopic study, the first visible changes were observed in the membrane three min after inoculation. The whole surface of the membrane became covered with a fuzzy adherent substance which was supposed to be an antigen-antibody complex. Soon after this the pellicular microtubules disappeared, but the axonemal microtubules remained intact for more than 30 min. The kinetoplastids have at least four functionally distinct groups of microtubules (pellicular, cytopharyngeal, axonemal and nuclear spindle types). The chemical differences of these microtubules are unknown, but this phenomenon seems to provide a convenient way for studying the chemical natures of these different microtubules. Subsequent changes showed that the mitochondrial membrane was more resistant than those of the nucleus, the Golgi-apparatus and the ER. It is unknown whether the structures projecting from the membrane are related to the circular lesions seen by negative staining. The projecting structures seemed to develop into small vesicles and separate from the membrane. Therefore, the vesicles around the membrane had the same structure as the plasma membrane. The circular lesions are structurally similar to the membrane lesions induced by the immunolytic reaction dependent on complement (Humphrey, 1969). These results suggest that a certain component of normal serum first reacts with a surface substance of epimastigotes and produces circular lesions in the membrane with the aid of complement, and then further destruction occurs as a result of osmosis.

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