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ULTRA-STRUCTURAL CHANGES IN THE KINETOPLAST OF *TRYPANOSOMA CRUZI* DURING TRANSITION OF FORM IN VITRO[1]

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SUMMARY In *Trypanosoma cruzi*, the fibrils of the kinetonucleus are uncoiled in the trypanosome (T) form, but coiled in the crithidia (C) form. Coiling occurs during the transition from the T to the C form. These fibrils are fixed on a plate-shaped structure that divides during replication of the kinetonucleus. This structure is designated as the ' basement plate '. Experiments on *T. gambiense* showed that the envelope membrane of the kinetoplast is destroyed, but the membrane adjascent to the basement plate is somewhat resistant to *p*-Rosaniline treatment.

Microtubules of the basal body were found connecting the flagellum and the kinetoplast.

INTRODUCTION

Trypanosoma cruzi, an agent of Chagas' disease, has three main forms during its life cycle, namely, trypanosome (T), crithidia (C) and leishmania (L) forms. The T form which is incapable of multiplication is found extracellularly in the plasma of infected individuals. The L form is found in cells of mesenchymal origin. The C form, an intermediate state in transition from the L to the T form, appears in culture media and in the invertebrate host, *Triatoma*.

The ultra-structure of this parasite has been studied extensively (Meyer and Porter, 1954; Meyer et al., 1958; Meyer and Queiroga, 1960; Schulz and MacClure, 1961; Sanabria, 1963, 1964, 1966), and a correlation between the morphological types and phases of the growth cycle was made by Camargo (see review, Weinman, 1968). However there are no reports on changes in the ultra-structure of the kinetoplast during the life cycle.

This study was on changes in the ultra-structure of the kinetoplast of *T. cruzi* during transition from the T to the C form.

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MATERIALS AND METHODS

A strain of *T. cruzi* obtained from N. I. H., Bethesda, Maryland, U.S.A., and *T. gambiense* (Wellcome strain) were used in this study. The latter was the same strain used by Inoki et al. (1960 and 1961) for their genetical and immunological studies. The T forms (blood forms) collected from infected mice were transferred to diphasic medium and cultured at 25 C. The technique for electron microscopy was identical with that reported by Inoki and Ozeki (1969). Parasites were taken from the diphasic medium or infected mice at desired times and fixed at 4 C for one hour in phosphate buffer (pH 7.4) containing 2.5% glutaraldehyde. The organisms were collected by centrifugation at 2,000 rpm for 15 minutes. The parasites formed a film on centrifugation were removed with a fine glass rod and washed for one hour with isotonic buffer. Then it was stored at 4 C overnight in the same isotonic buffer. The materials were post-fixed at 4 C for 1 hr 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, 1963). Micrographs were made in a Hitachi 11B electron microscope.

RESULTS

We have shown that when the T form of *T. cruzi* is transferred from mice to the diphasic medium of Phillips (1961), it changes to the C form in about 90 hr. Employing this transition system, we followed this process. In the first step, it was clearly demonstrated that the kinetonucleus is uncoiled in the T form (Fig. 1 and 2). The uncoiled fibrils of the kinetonucleus gradually change to the compact, coiled fibrils characteristic of the C form (Fig. 3 to 14), and large spaces are formed inside and outside the kinetoplast as the result of coiling of the kinetonucleus (see Fig. 7, 8, and 9).

Fig. 3 to 8 show organisms taken 24 hr after transfer from mouse blood to the diphasic medium, and Fig. 9 to 14 show parasites 90 hr after the transfer. From these photographs, it is evident that the transition from the T to the C form is always accompanied by a morphological change of the kinetonucleus. The kinetonucleus gradually coils up, and then the vacuoles decrease in size. Finally the kinetonucleus becomes compact with no spaces, and is morphologically indistinguishable from that of the C form. It was also noticed that during these processes some coiled kinetonuclei start to duplicate before transition to the C form is complete (see Fig. 9).

A plate-like structure can be seen on the inside of the membrane enveloping the kinetoplast from which the kinetonucleus may arise (see Fig. 2, 3, and 4). We named this the 'basement plate'. Similar observations were made on the blood form (T form) of *T. gambiense*, and a basement plate was also detected in its kinetoplast (see Fig. 15), although it was not so distinct as in *T. cruzi* (see Fig. 15 and 16). This may be because the kinetonucleus is not uncoiled in the T form of *T. gambiense*. As shown in Fig. 15, it was also found that the basement plate divides simultaneously with replication of the kinetonucleus.

In 1969, Inoki et al. reported that on treatment with *p*-Rosaniline, the envelope membrane of *T. gambinese* is lost while it remains intact in *T. evansi*. The change in the ultra-structure of the kinetoplast of the blood form of *T. gambiense* (in mouse) was examined 4 hr after treatment with *p*-Rosaniline (5 mg/Kg/b.w.). Fragmentation of the kinetonucleus and disappearance of the envelope membrane occurred, but the membrane adjacent to the basement plate was somewhat resistant (see Fig. 16).

As shown in Fig. 7, two fibrils were found connecting the flagellum to the kinetoplast.

DISCUSSIONS

There are several reports on the ultra-structure of *T. cruzi*. The preliminary report of Meyer

and Porter (1954) on electron microscopic studies on the C and T forms of *T. cruzi* grown in NNN medium, was confined to non-sectioned preparations. Employing the ultra-thin section technique, Meyer et al. (1958) and Meyer and Queiroga (1960) respectively reported observations on *T. cruzi* in tissue culture and in blood agar medium. Schulz and MacClure (1961) also studied *T. cruzi* in blood agar medium and demonstrated the fine structures of the fibrils in the periplast and kinetoplast. Recently, Sanabria (1963, 1964, 1968) published a series of papers on electron microscopic studies on *T. cruzi* in mouse brain and myocardium. These reports give detailed descriptions of the morphological characters of various organelles, including the nucleus, kinetoplast, blepharoplast, Golgi apparatus, flagellum, periplast and ribosomes. However, the change in the ultra-structure of the kinetonucleus during transition from the T form to the C form did not receive attention.

This work was on the morphology of the kinetonucleus in the different forms of *T. cruzi*, because the kinetoplast is considered to contain the DNA. It was found that the kinetonucleus is uncoiled in the T form (see Fig. 1 and 2), but coiled in the C form (see Fig. 14). The process of coiling of the kinetonucleus during the transition of form on transfer to diphasic medium was followed by electron microscopy (see Fig. 3 to 13). Kinetonuclear division was observed only when fibrils of the kinetonucleus were coiled. This division does not seem to require complete conversion to C form, but can occur when coiling of kinetonuclear fibrils has proceeded to a certain stage (Fig. 8). In contrast to the T form of *T. cruzi*, it is known that the blood forms (T forms) of other species of the genus *Trypanosoma* are usually capable of duplication. In these, the T form has coiled kinetonuclear fibrils. Thus, only the coiled kinetonucleus seems able to divide.

A plate-like structure, from which kinetonuclear fibrils appear to arise, was demonstrated here for the first time inside the envelope membrane of *T. cruzi* and *T. gambiense*. This structure divided at the same time as the kinetoplast. Accordingly it was named the 'basement plate'. The roles of the basement plate are not known, but it may fix the kinetonucleus in the right position in the kinetoplast relative to the basal body of the flagellum, and/or may be involved in synthesis of kinetonucleus fibrils.

As shown in Fig. 7, two microfibrils (tubulae) were observed connecting the base of the flagellum to the kinetoplast (cf. rhizoplast; Pitelka, 1963). The term 'kinetoplast' means an organelle that gives energy for flagellar movement. However, this function has not yet been proved experimentally. Anderson and Ellis (1965) stated that if the kinetoplast produces energy yielding material for use in flagellar or pellicular movement, this material must diffuse through the double membrane, since no micropores have been observed. From studies on *Trypanosoma mega*, however, Steinert (1960) considered that the kinetoplast provides genetic information for synthesis of mitochondrial enzymes. Later, the concept of the kinetoplast as a 'mother mitochondrion' (Ris, 1962) became widely accepted, and the original view that the kinetoplast controls flagellar movement, has gradually changed. However, our results, suggest that the original view may be revived.

FIGURE 1. *The T form of T. cruzi in mouse blood. The kinetonuclear fibers are uncoiled and thicker than typical coiled ones. The arrow indicates the basement structure [so-called ' basement plate ' (Bp)] on the inside of the kinetoplast envelope.*

Wc, white blood cell; en, kinetoplast envelope

FIGURE 2. *Region of the kinetonucleus (Kn) of Fig. 1, at higher magnification. The basement plate and kinetonuclear fibers are clearly seen.*

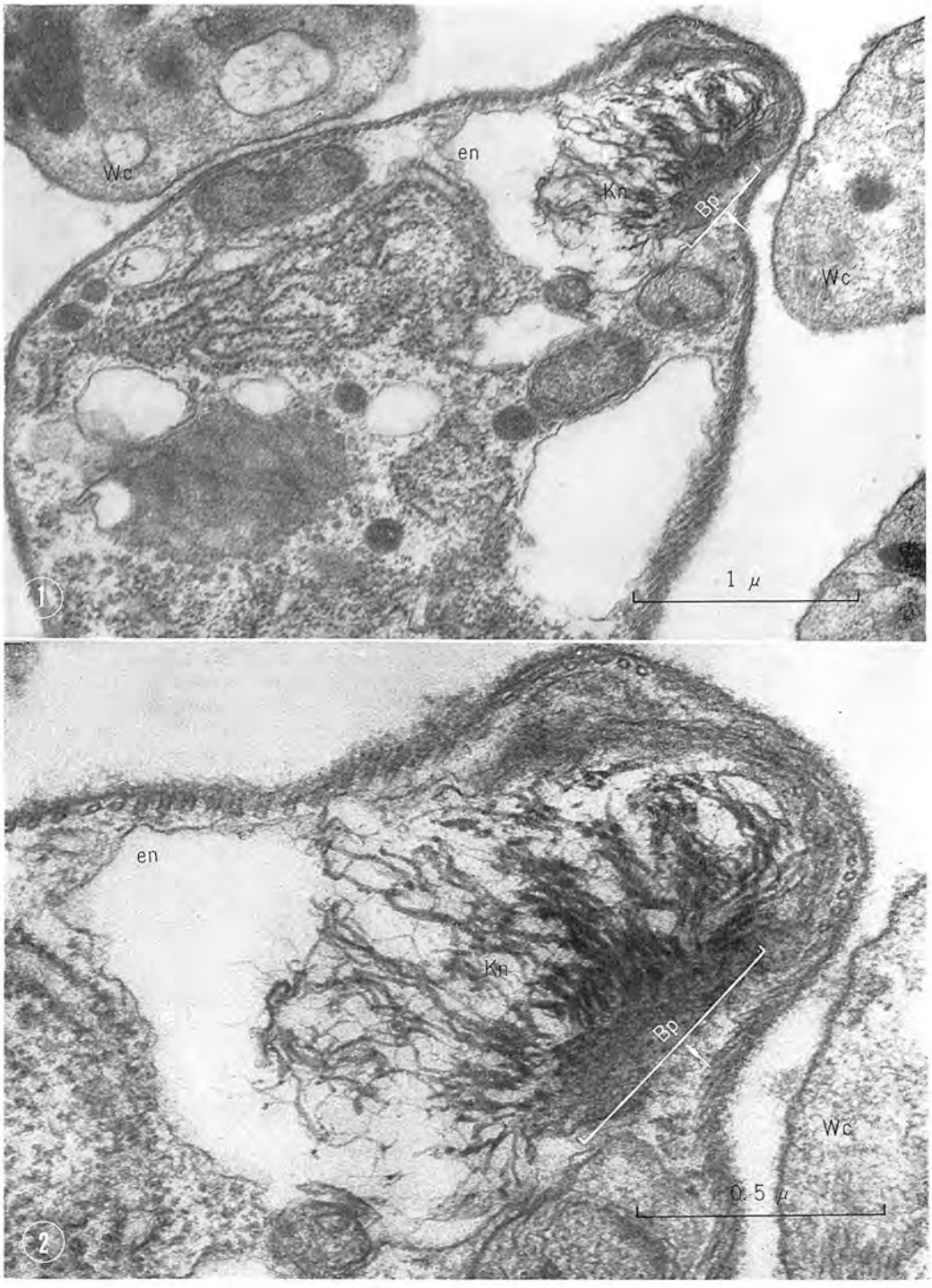

Wc
en
Kn
Bp
Wc
1 μ
1
en
Kn
Bp
Wc
0.5 μ
2

FIGURE 3 to 14. *Structural change in the kinetonucleus of the blood form of T. cruzi on transfer to diphasic medium. The samples were taken 24 hr (Fig. 3 to 8) and 90 hr (Fig. 9 to 14) after transfer to diphasic medium.*

FIGURE 3 to 6. *Show kinetonuclei (Kn) which are still uncoiled. The spaces inside (Si) and outside (So) the kinetoplast envelopes are seen in Fig. 4 and 5. Fig. 5 and 6 show basement plates (Bp) probably connected with the kinetonuclei. In Fig. 6, the kinetonucleus is close to the nucleus (N). bb, basal body (or blepharoplast); en, kinetoplast envelope*

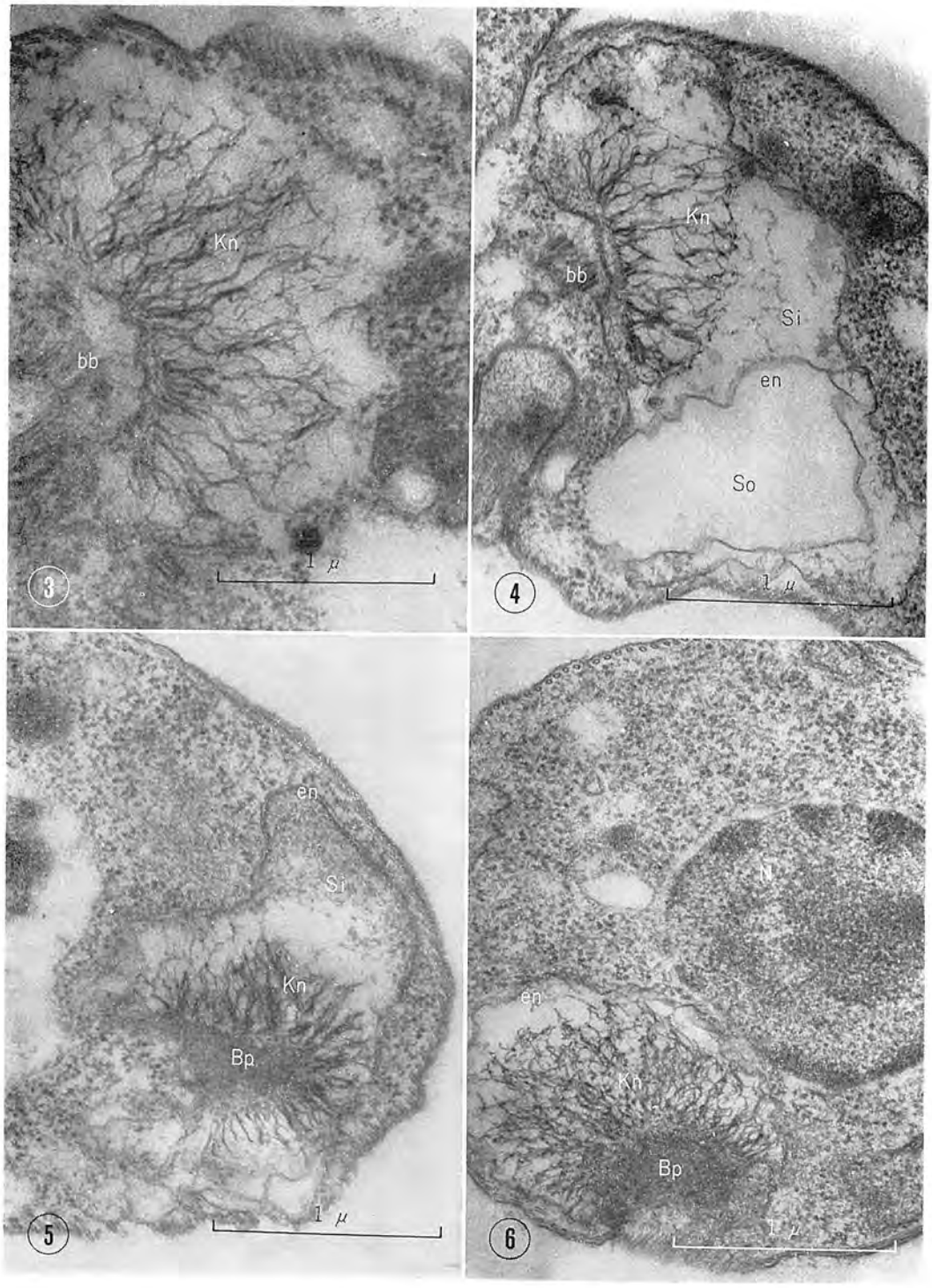

Kn
bb
1 μ
3
Kn
bb
Si
en
So
1 μ
4
en
Si
Kn
Bp
1 μ
5
N
en
Kn
Bp
1 μ
6

FIGURE 7 and 8. *Kinetoplasts 24 hr after transfer to diphasic medium. Fig. 7 shows an uncoiled kinetonucleus (Kn), and big spaces both inside (Si) and outside (So) the kinetoplast envelope (en). Two microtubules can be seen connecting the envelope membrane of the kinetoplast to the base of the flagellum (arrows). In Fig. 8, the kinetonucleus is coiled but spaces, Si and So, are still seen. Thread-like structures are seen in space Si. bb, basal body (or blepharoplast)*

FIGURE 9. *The divided kinetoplast and nucleus (N) of T. cruzi 90 hr after transfer to diphasic medium. Two kinetonuclei (Kn) are located adjacent to the nucleus. Coiled kinetonuclei and spaces are also seen. Thread-like structures are seen in the inside spaces.*

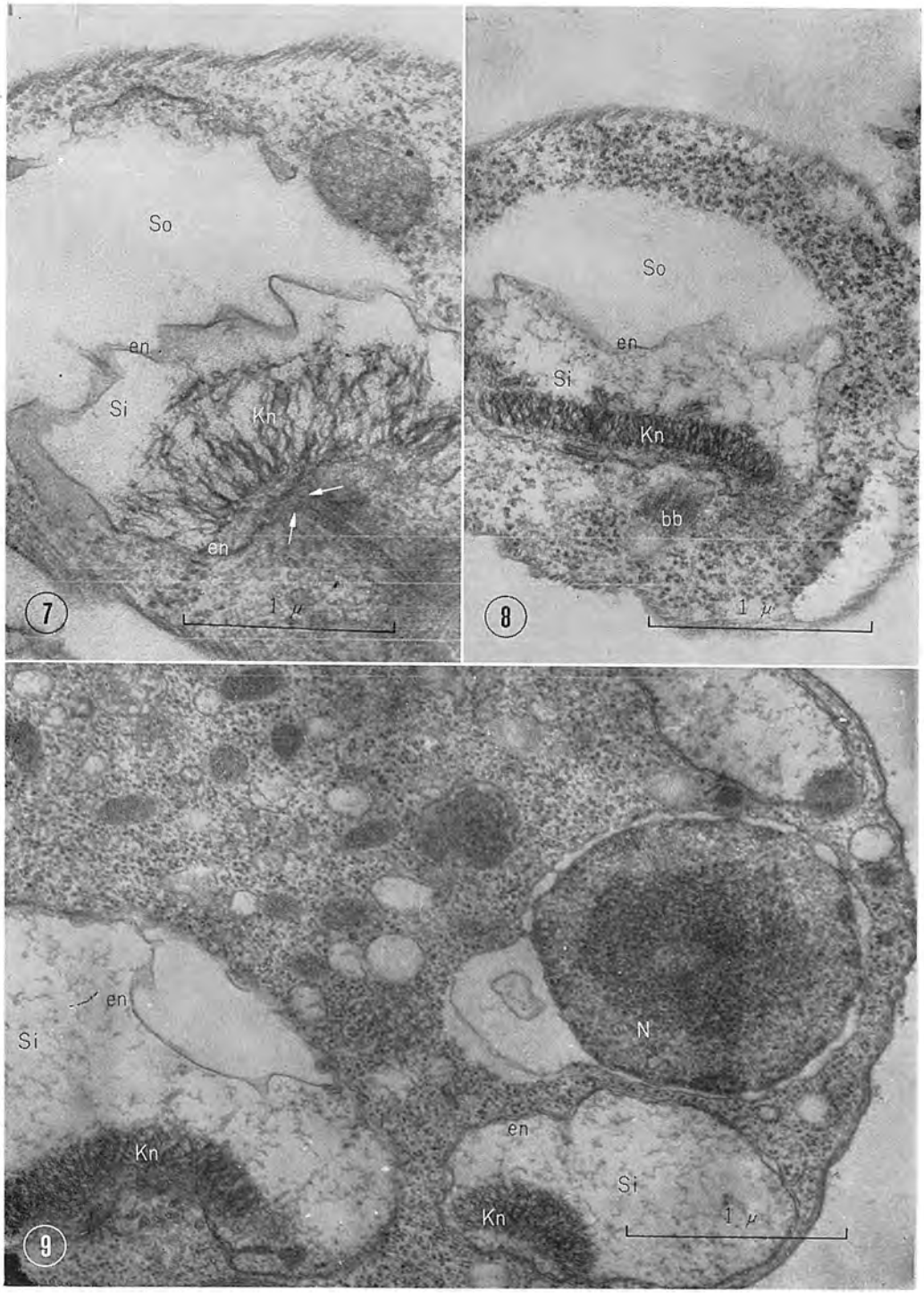

So
en
Si
Kn
en
7
1 μ
So
en
Si
Kn
bb
8
1 μ
en
Si
N
en
Kn
Si
Kn
9
1 μ

FIGURE 10 to 13. *Kinetoplasts and nuclei of T. cruzi 90 hr after transfer to diphasic medium. The kinetonuclei (Kn) are close to the nuclei (N). The spaces inside (Si) and outside (So) the kinetoplast envelope (en) are smaller than previously shown. bb, basal body(or blepharoplast)*

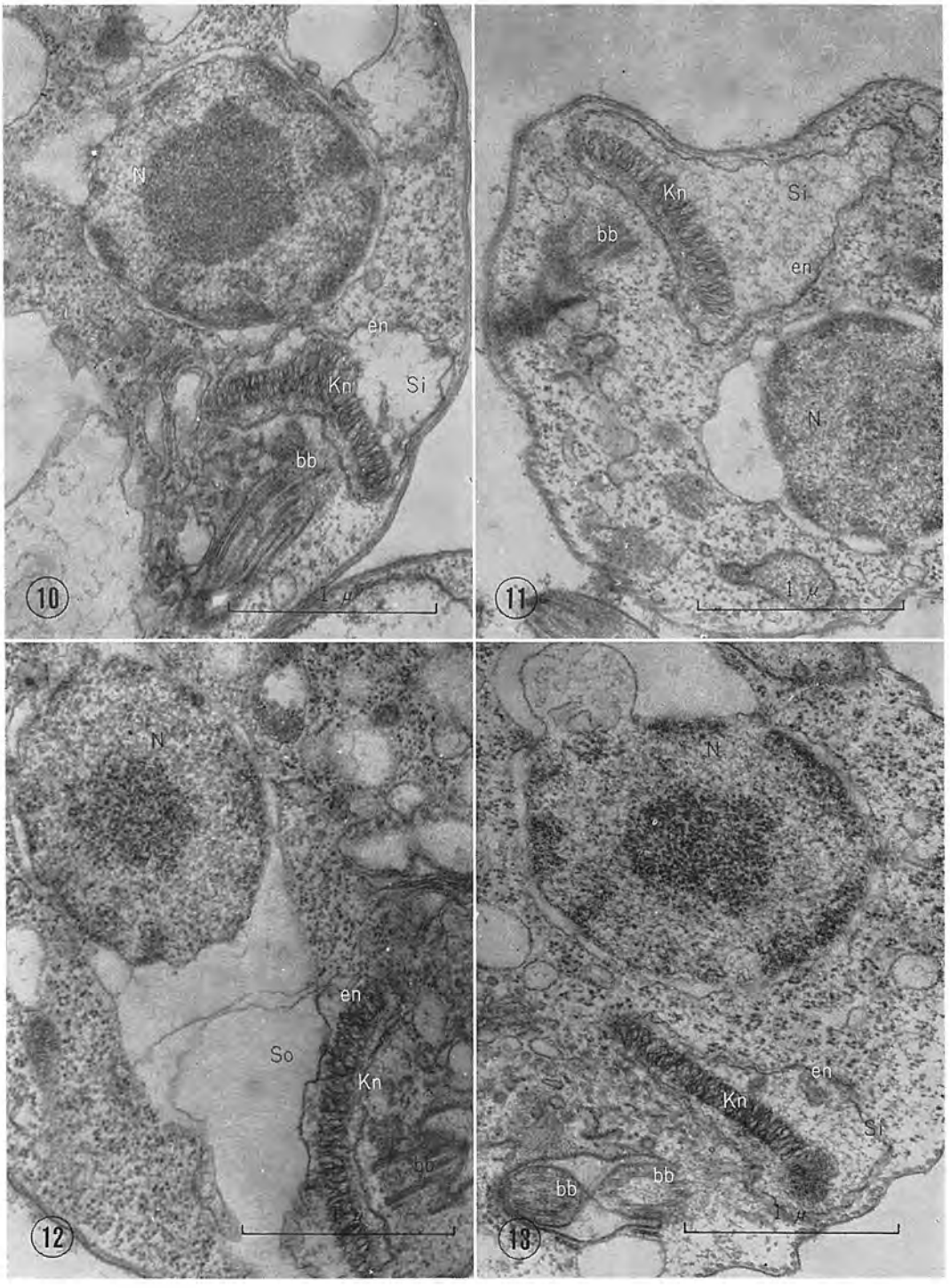
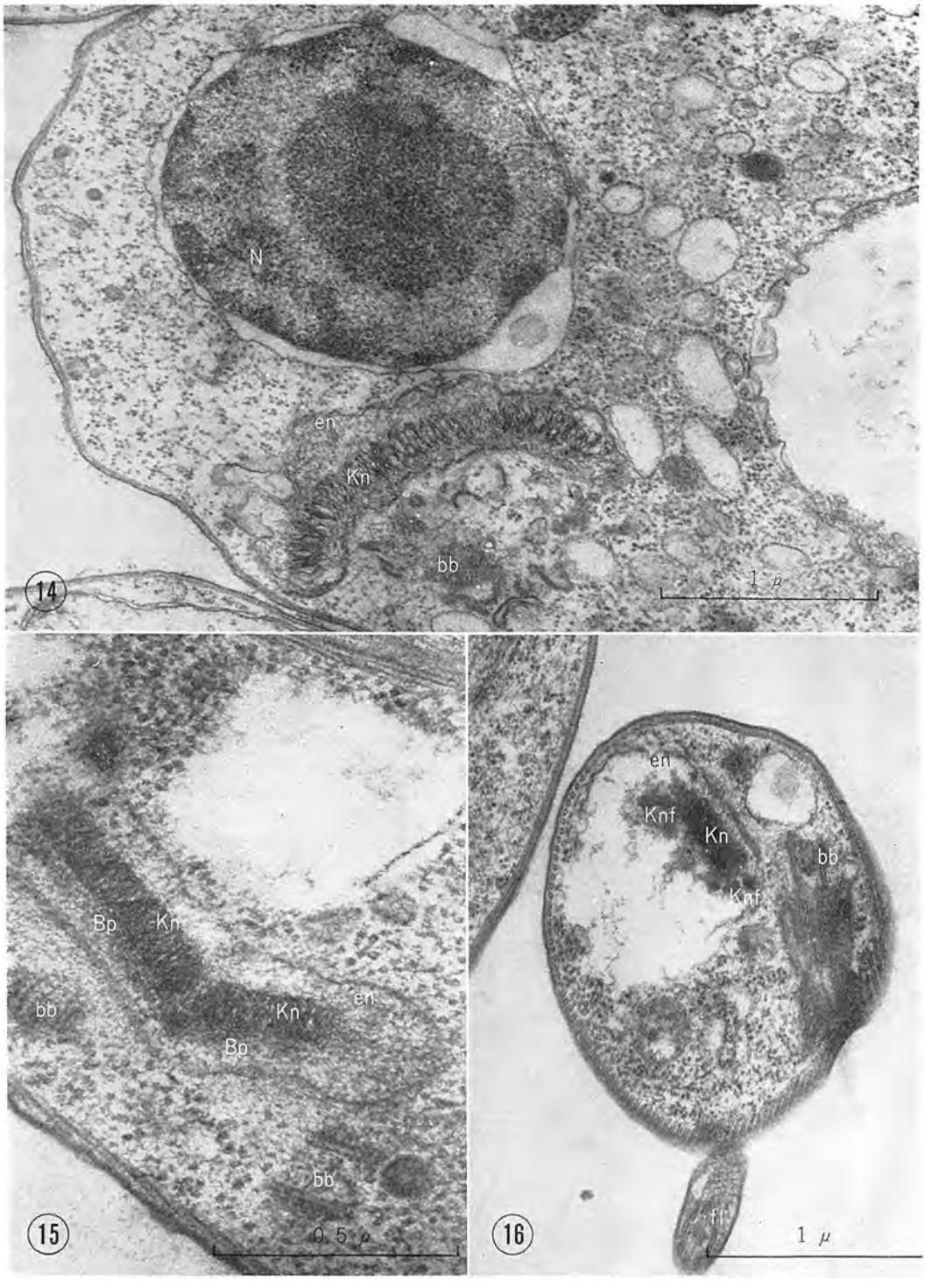

N
en
Kn
Si
bb
1 μ
10
Kn
Si
bb
en
N
1 μ
11
N
en
So
Kn
bb
1 μ
12
N
en
Kn
Si
bb
bb
1 μ
13

FIGURE 14. *The crithidia form (C form) of T. cruzi 90 hr after transfer to diphasic medium. No space is seen in the kinetoplast. bb, basal body (or blepharoplast)*

FIGURE 15. *Dividing kinetonucleus (Kn) of T. gambiense. The figure also shows a duplicating basement plate (Bp).*

FIGURE 16. *Kinetonucleus (Kn) of T. gambiense after exposure to p-Rosaniline in mouse. The kinetonucleus has fragmented (Knf) and the kinetoplast envelope (en) has disappeared, but the envelope membrane close to the basement plate is uninjured. en, kinetoplast envelope*


N
en
Kn
bb
1 μ
14
Bp
Kn
bb
Kn
en
Bp
bb
0.5 μ
15
en
Knf
Kn
bb
Knf
fl
1 μ
16

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