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Study of *Leishmania donovani* with special Reference to the Kinetoplast, Mitochondria, and Golgi Zone by Electron Microscope employing the Thin Section Technique

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SUMMARY

The fine structures of the kinetoplast, mitochondria, and Golgi zone in *Leishmania donovani* was studied by electron microscope. 1. The kinetoplast is an independent cytoplasmic particle surrounded by a thin layer of ca. 8-15 mμ in thickness. It's diameter varies, depending on the plane of the section, being 600-870 mμ in length and 120-435 mμ in breadth. Inside this particle, dense striations (ca. 15 mμ breadth and ca. 20-45 mμ length) run parallel with the major axis. 2. The mitochondria are variably shaped and carry the '*Cristae mitochondriales*' of Palade, which are ca. 220-850 mμ in length and ca. 110-300 mμ in breadth. The limiting membrane of 8-20 mμ. thickness was very clearly visible. 3. A structure similar to the Golgi zone of rat spermatids described by Clermont (1956) was demonstrated in some section. This contained several spherical vesicles of variable size (ca. 100-125 mμ × 50 mμ).

INTRODUCTION

As a part of our genetic studies on pathogenic protozoa (Inoki, Kitaura, Nakabayashi, and Kurogochi; 1952; Inoki, 1952; Inoki, Kitaura, Kurogochi, Osaki, and Nakabayashi, 1952; Inoki and Hamada, 1953; Inoki, 1957), observations on their fine structure have been made in this laboratory by electron microscope. In the previous paper (Inoki *et al.*, 1957), the constitution of the flagellum of *Leishmania donovani* was described. This paper is on the fine structure of the cytoplasmic particles and Golgi zone in *L. donovani*.

It is known that the kinetoplast is a self-duplicating cytoplasmic entity regularly found at the base of the flagellum in protozoa. It has recently become of interest in protozoan genetics (Inoki, 1956). Accotdingly, the fine structure of the kinetoplast was studied in most detail, and the other cytoplasmic bodies in less detail.

Materials and Methods

A stock culture (Strain Kitagawa) of *Leishmania donovani* was employed for

An outline of this work was reported at the 27th Annual Meeting of Japanese Parasitologists in Gifu City in May, 1956, and at the 29th Congress of Japanese Genetics Society in Sapporo City in September, 1957.

this series of experiments. Cells were grown in Tanabe's monophasic medium* for 7 to 14 days and collected by centrifuging at 3,000 r.p.m. They were washed three times with physiological saline and then fixed in 1% buffered osmic acid (pH 7.4) as reported by Inoki *et al.* (1957). The fixed preparations were dehydrated in alcohol before being embedded in *n*-butyl-, *n*-methyl-metacrylate mixture (20 : 3 v/v). Thin sections were prepared in a Spencer Model 820 or a Model RU (Japanese) microtome and examined in a Hitachi Model HU-6 electron microscope at 50 KV.

* Tanabe's Monophasic (Liquid) Medium

Aqua distillata	one part	Solution A Shaken throughly to complete hemolysis
Defibrinated rabbit blood	one part	
Sodium citrate	12 gm.	Solution B
Sodium chloride	10 gm.	
Aqua distillata	950 ml.	

Equal quantities of solutions A and B were mixed. The mixture was dispensed in 5-10 ml. aliquots in small tubes.

RESULTS

1. Kinetoplast (or Parabasal body)

In the preceeding paper (Inoki *et al.*, 1957), a mention was made of the suspected kinetoplast at the base of the flagellum. In this study however the fine structure was successfully demonstrated more precisely or clearly. Usually the kinetoplast appeared as a long-rod, crescent, or occasionally triangular shaped body in the section, with no visible connection with the flagellum. This structure, 600-870 mμ long and 120-435 mμ wide, was surrounded by a thin layer of ca. 8-15 mμ in thickness. It should be stressed here that there was a peculiar dense striation parallel with its major axis which regularly appeared in each kinetoplast. On closer examination, the striation was found to consist of granular bodies of ca. 15 mμ in breadth and ca. 25-45 mμ in length. (See Figs. 1, 2, 3, 4, 5, and 6.)

2. Mitochondria

The mitochondria was demonstrated in the cytoplasm. It was rod shaped, oval, round, or crescent shaped depending on the plane of the section. It was surrounded by a clearly defined limiting membrane of ca. 8-20 mμ in thickness. The mitochondriae were ca. 220 mμ in length and ca. 110-300 mμ in breadth. Inside the mitochondria, the so called '*Cristae mitochondriales*' of Palade were seen. The size and number of the cristae were variable and were ca. 8-33 mμ in breadth and ca. 30-250 mμ in length. Only 5 to 7 cristae were counted in each section of the mitochondria. (See Figs. 2, 3, 5, and 6).

3. Golgi zone

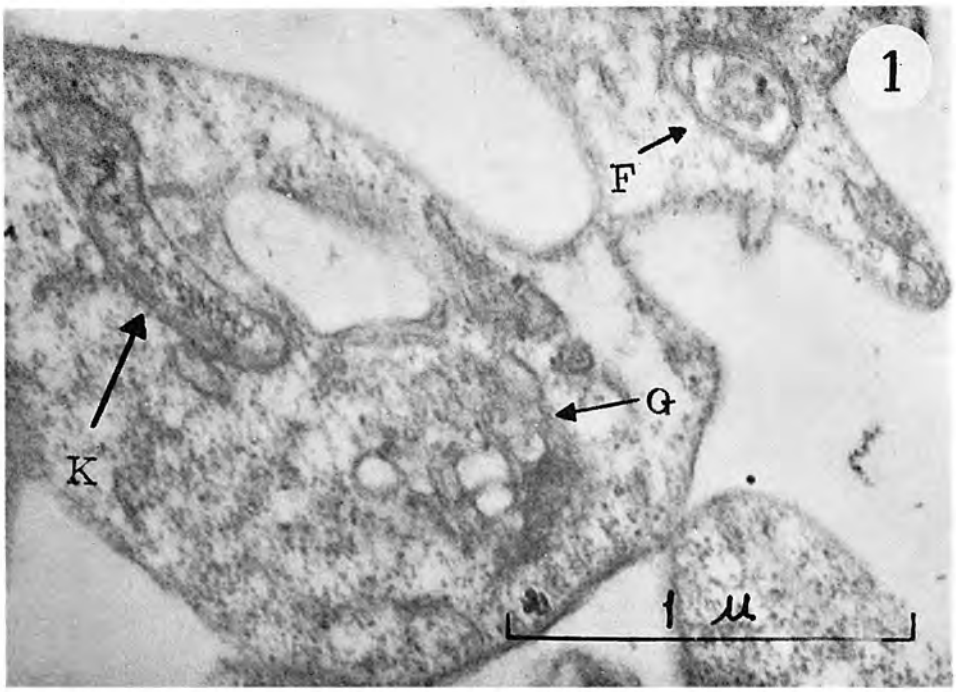
Structures similar to the Golgi zone in rat spermatids reported by Clermont (1956) were found in some sections. They contained several spherical vesicles of various sizes (ca. 100 mμ × 125 mμ—50 mμ × 50 mμ). However no granular compoments were observed (See Figs. 1, 2, and 3.).

DISCUSSION

Employing the thin section technique, Inoki *et al.* (1957) have already reported on the fine structure of the flagellum on *Leishmania donovani.* However in that report few observations were made on the cytoplasmic particles, the kinetoplast (parabasal body) and the mitochondria. The following year, a similar report (Pyne, 1958) appeared on the culture forms of *L. donovani.* This paper only covered observations of the cell surface, nucleus, mitochondria, and flagellum, and did not describe the kinetoplast.

As already mentioned, the kinetoplast has recently become important in cytoplasmic genetics of flagellated protozoa (Inoki, 1957). On staining by ordinary methods, it develops fine granules in the cytoplasm. Using the thin section technique, however, it was possible to show that the structure of the kinetoplast is very complicated, and probably has no morphological connection with the base of the flagellum. Recently, Meyer *et al.* (1958) studied the ultrastructure of the kinetoplast in *Trypanosoma cruzi.* They used an electron microscope, but the microphotographs in their paper do not show the inner structure of the striation in the kinetoplast. In this study, however, it should be noted that the striation were demonstrated very clearly and precisely. It is reasonable to assume that the fine structure of the kinetoplast is similar in different species of flagellated protozoa. That is that it is an independent cytoplasmic particle distinctly limited by a membrane and contains a dense striation running parallel to the longitudinal axis. Since the kinetoplast is known to contain DNA, it remains a question of the future which part of the kinetoplast carries it. Another problem is how the subcomponents of the kinetoplast behave during cell division.

Mitochondria have now been demonstrated in several species of protozoa by the ultra-thin section technique. In non-pathogenic protozoa, typical mitochondria have been found in *Paramecium caudatum* (Powers, Ehret, and Roth, 1955), *Paramecium aurelia* and *Paramecium bursaria* (Powers, Ehret, and Roth, 1955), *Paramecium multimicronucleatum* (Sedar and Porter, 1955), *Euplotes patella* (Powers, Ehret, Roth, and Minik, 1956) *Tetrahymena pyriformis* (Sedar and Rudzinska, 1956), *Tokophyra infusionum* (Sedar and Rudzinska, 1956), *Amoeba proteus* (Sedar and Rudzinska, 1950), and some others. In parasitic protozoa, only a few reports are available in the literature on the fine structure of the mitochondria ; Anderson (1955) stated that spherical mitochondria are seen in thin sections of *Trichomonas muris.* Rudzinska and Trager (1957) reported the demonstration of mitochondria in *Plasmodium lophurae* employing a similar technique. Inoki *et al.* (1957), and Pyne (1958), also demonstrated the fine structure of the mitochondria in *Leishmania donovani.* Judging from the microphotographs in their papers however (with the exception of) mitochondria in *Leishmania donovani* these results are still dubious. Moreover Anderson's result (1956) on *Trichomonas muris* is quite inconsistent with that of Inoki et al.'s results on *Trichomonas vaginalis* (1958), *Trichomonas foetus* (Inoki *et al.*, unpublished), and *Trichomonas tenax* (Inoki *et al.*, unpublished). The latter workers could find no mitochondrial structure such as described in metazoan tissues in the Genus *Trichomonas.* In this paper, Golgi zones have been demonstrated, but much more work is necessary before they can be conformed to be true Golgi zone.



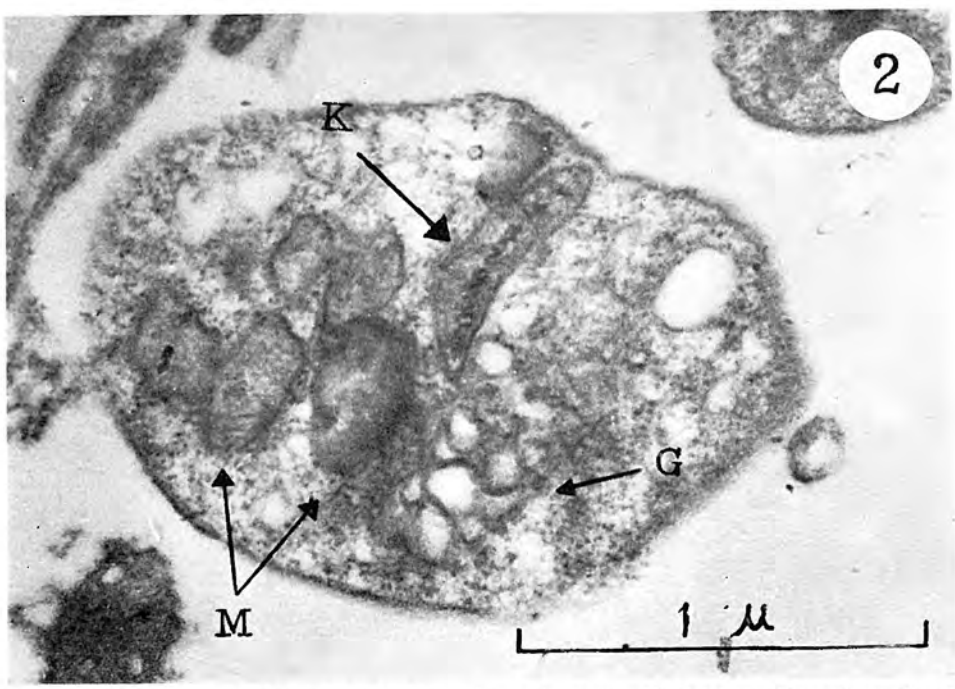
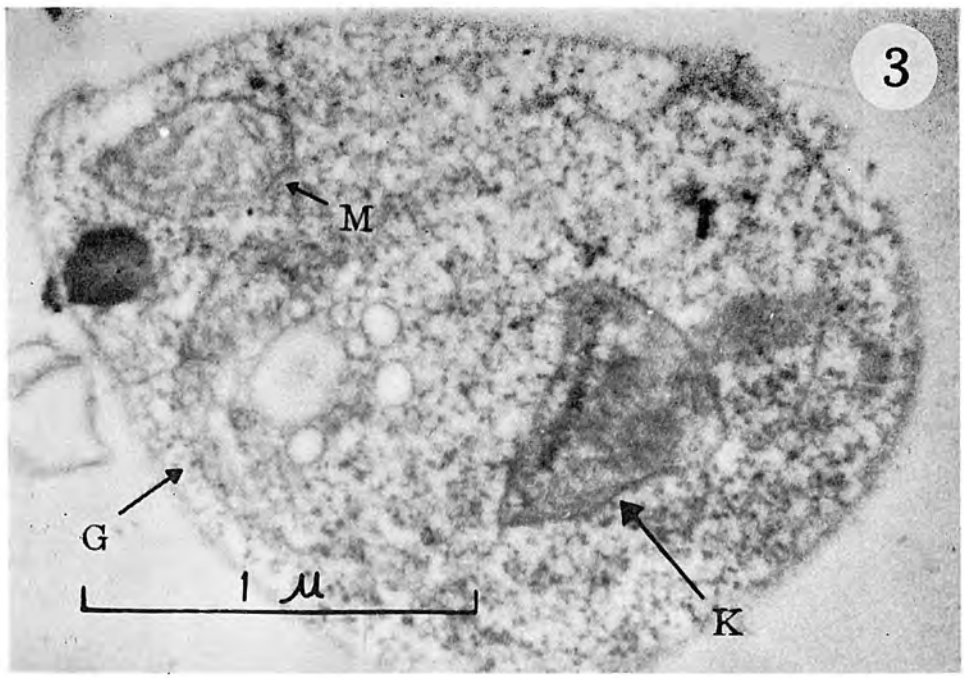


Fig. 1. An ultra-thin section of *Leishmania donovani* showing kinetoplast (K), Golgi zone (G); and flagellum (F).
Fig. 2. An ultra-thin section of *Leishmania donovani* showing kinetoplast (K) and mitochondria (M).

(INOKI *et al.*)



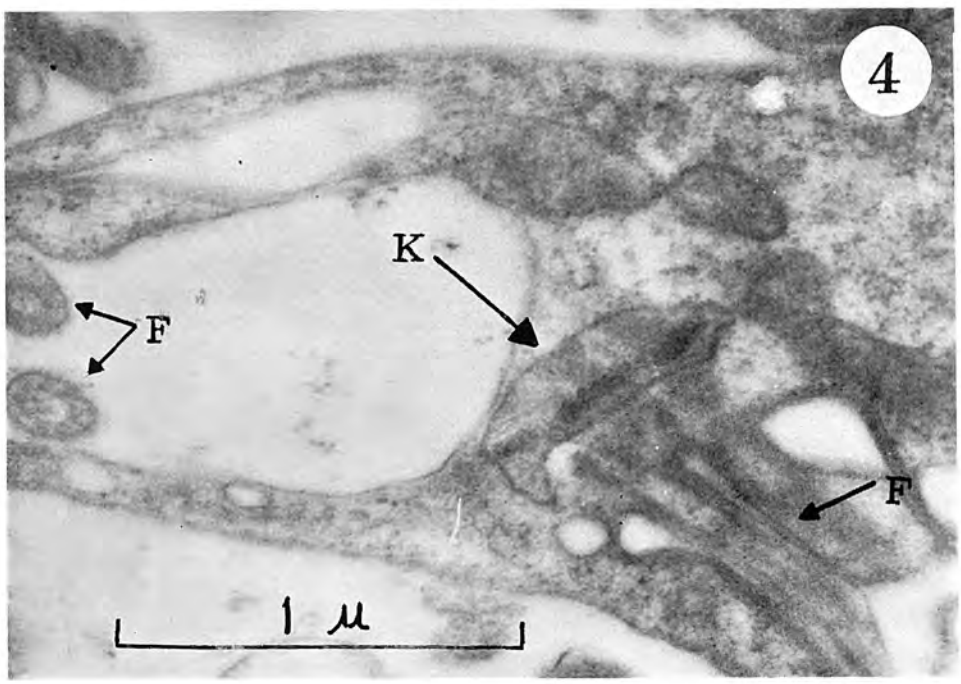
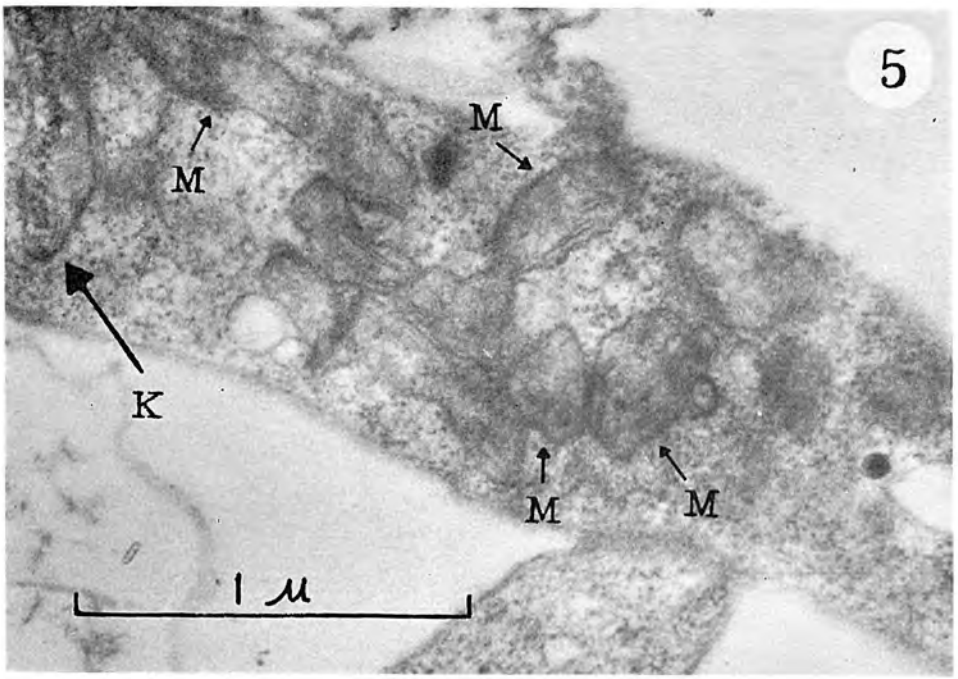


Fig. 3. An ultra-thin section of *Leishmania donovani* showing kinetoplast (K), Golgi zone (G), and mitochondria (M).

Fig. 4. An ultra-thin section of *Leishmania donovani* showing kinetoplast (K) and flagella (F).

(INOKI *et al.*)



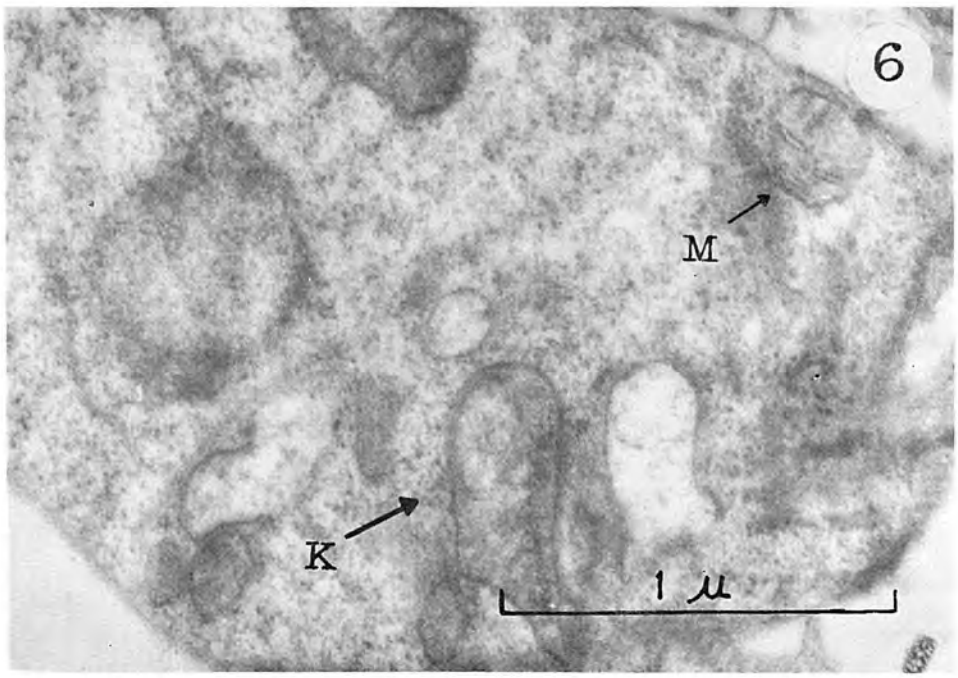


Fig. 5. An ultra-thin section of *Leishmania donvani* showing kinetoplast (K) and mitochondria (M).

Fig. 6. An ultra-thin section of *Leishmania donvani* showing kinetoplast (K) and mitochondria (M).

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