MITOCHONDRIA ASSOCIATED WITH THE KINETONUCLEUS OF *TRYPANOSOMA MEGA*

M. STEINERT. From the Laboratoire de morphologie animale, Université libre de Bruxelles, Belgium, and Department of Pathology, Albert Einstein College of Medicine, New York. Dr. Steinert's permanent address is Institut pour la Recherche Scientifique en Afrique Centrale, Centre de Recherche du Katanga, Elisabethville, Katanga Republic

Neither the function nor the name of the deoxyribonucleic acid– (DNA) containing body found near the base of the flagellum in trypanosomids seems to have been definitively established. Adopting Wenyon's (1) and Grassé's (2) nomenclature, we called this structure "parabasal body" in former publications (3). Since the recent observations of Grassé (4) and Grassé and Carasso (5), it appears necessary to reserve this term for the Golgi apparatus of some flagellates and to name the DNA-containing particle kinetoplast or kinetonucleus in accordance with most authors. The role that this particle plays in the physiology of the trypanosome is unclear. Viable strains without kinetonuclei are known, but only in the most specialized and mammalian host-dependent species and they have never been maintained in *in vitro* cultures. This might be seen as evidence for an im-

Received for publication, May 17, 1960.

All sections are of the crithidia form of *T. mega*.

FIGURE 1

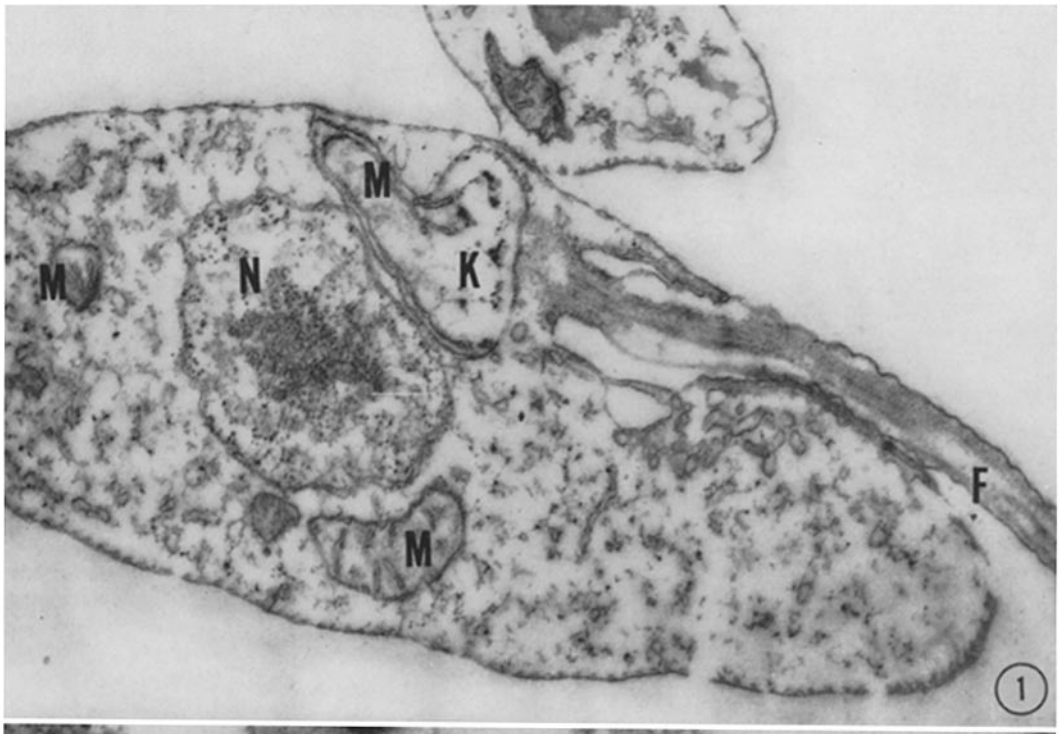
Longitudinal section stained with lead hydroxide. × 32,000.

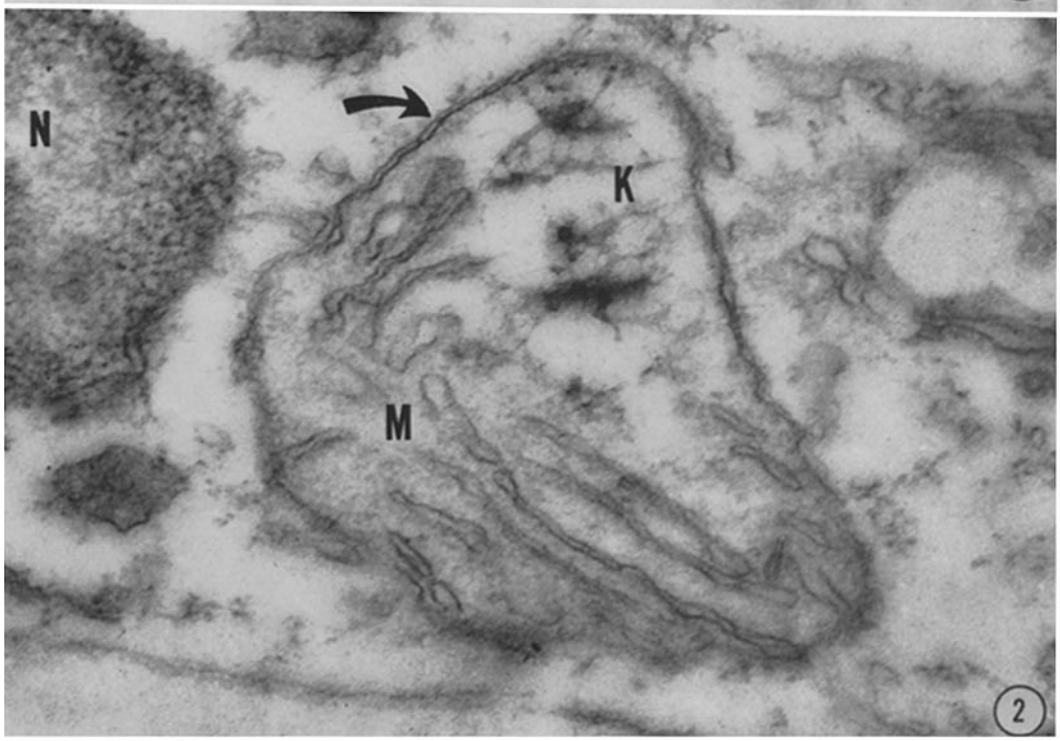
This section shows the nucleus (*N*), kinetonucleus (*K*), and flagellum (*F*). The mitochondria (*M*) are seen free in the cytoplasm or associated with the kinetonucleus.

FIGURE 2

Oblique section stained with lead hydroxide. × 70,000.

Note the absence of any separation between the DNA-containing kinetonucleus (*K*) and the mitochondrion (*M*). It is evident (arrow) that the same outer membrane encloses both structures. Part of the nucleus is seen at *N*.





portant function of the kinetonucleus, a function that may be essential only in the crithidia form of trypanosomes.

A previous study of this organelle in the crithidia of *T. mega* led to the conclusion that it synthesizes DNA (3). The present paper brings evidence of a structural and perhaps physiological relationship between the DNA-containing body and the mitochondria.

MATERIAL AND METHODS

Crithidias of the trypanosome, *T. mega*, grown *in vitro* in a bacto-tryptose medium (6), are collected by low speed centrifugation and are fixed in cold 1 per cent osmium tetroxide, buffered at pH 7.4 (7). Embedding is performed in a 9:1 mixture of *n*-butyl- and methyl-methacrylate. Polymerization, catalyzed at room temperature by benzoyl peroxide, is accelerated by UV irradiation. Sections, made with a Servall Porter-Blum microtome, are mounted on uncoated grids, then coated with carbon, and stained with lead hydroxide (8). In an alternate method, a 5 to 1 mixture of *n*-butyl- and methyl-methacrylate with 0.075 per cent uranyl nitrate (9) is used for embedding. Polymerization is catalyzed by benzoyl peroxide at 55°C. The sections, mounted on formvar-coated grids, are observed unstained. The sections are examined with either the Siemens Elmiskop I or RCA EMU 3B electron microscope.

OBSERVATIONS AND DISCUSSION

The kinetonucleus generally appears as a rounded body with its longer axis, approximately 1 micron in length, roughly perpendicular to the anteroposterior axis of the cell. It is bounded by a double membrane and within it is a fibrillar material presumed to contain DNA (3). A more or less similar appearance has been observed in the kinetonuclei of *T. equiperdum* by Anderson *et al.* (10) and of *T. cruzi* by Meyer *et al.* (11).

In favorable sections, it is seen that the kinetonucleus extends into structures with the typical features of mitochondria, including inner membranes or "cristae" (Figs. 1 to 4). There is considerable variability in the arrangement of these cristae. Generally, they have the orientation found in mitochondria of most other cells (Figs. 2 and 3), but it is not unusual to find concentrically arranged membranes (Fig. 4) resembling those described in *Plasmodium* by Rudzinska and Trager (12) and in spermatids by Grassé *et al.* (13) and by Fawcett (14). These different arrangements may be observed within the same mitochondrion. It is not clear at present to what extent these variations reflect developmental or physiological differences and to what extent they are due to the plane of sectioning. An extended statement of these and other observations will be published subsequently.

Preliminary experiments indicate that the kinetonucleus-associated mitochondria, like the others in the cytoplasm, give positive reactions for Janus green B *in vivo* and for DPNH-tetrazolium reductase activity in fixed smears.

The outer mitochondrial membrane is continuous with the outer kinetonucleus membrane, and there is no membrane or other structure separating the mitochondrial matrix from the interior of the kinetonucleus with its DNA-containing fibrillar material. The kinetonucleus of trypanosomes is known to be endowed with genetic continuity. Its possession of DNA, generally considered to store and transmit genetic information, makes its intimate association with mitochondria of considerable physiological significance. A more extensive investigation of the mitochondria in trypansomids and in other protozoa may extend our still

FIGURE 3

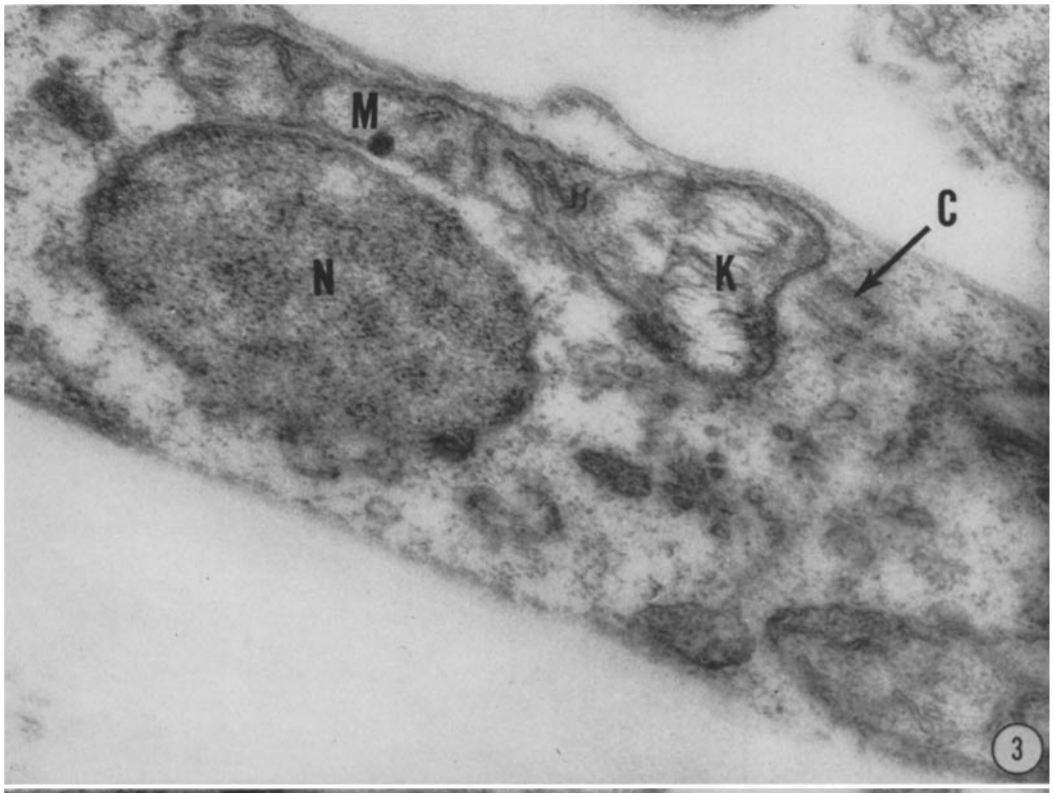
Longitudinal section, unstained. × 48,000.

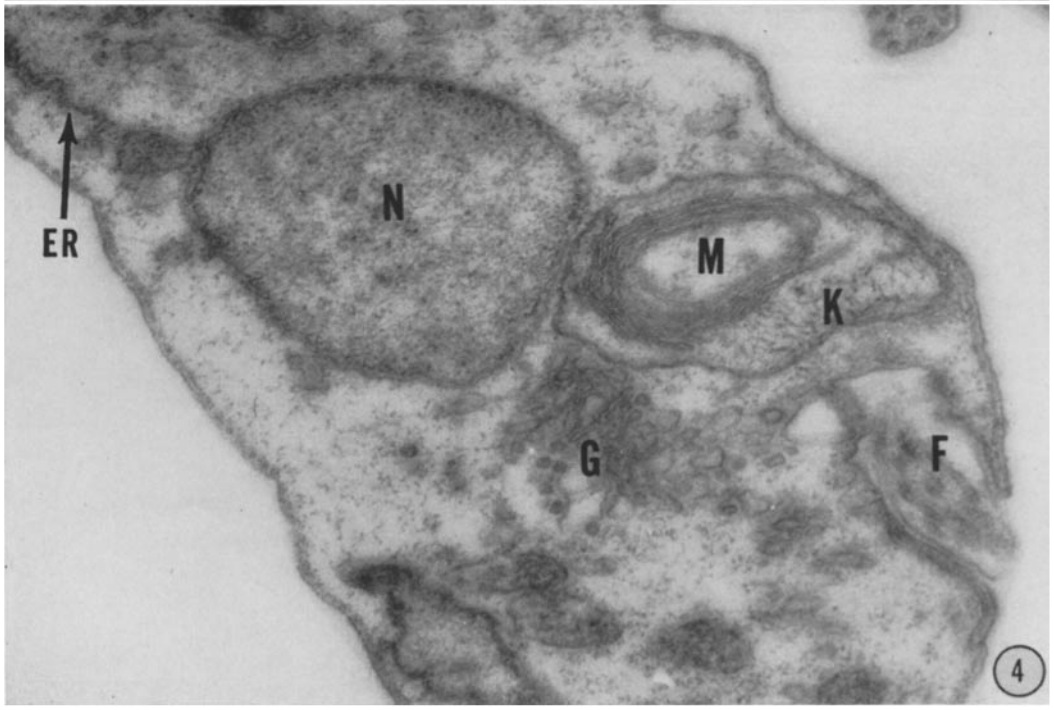
A large mitochondrion (*M*) with randomly oriented cristae is seen associated with the kinetonucleus (*K*). The section shows another mitochondrion in the lower right corner, the nucleus (*N*), and the centriole (*C*).

FIGURE 4

Oblique section, unstained. × 35,000.

The mitochondrion (*M*) associated with the kinetonucleus (*K*) contains inner membranes which appear concentric in this section. This section also shows the nucleus (*N*), endoplasmic reticulum (*ER*), flagellum (*F*), and Golgi apparatus (*G*).





limited knowledge of the ontogenesis of mitochondria. It is probable that future investigations will show the mitochondrial nature of unidentified bodies associated with the kinetonuclei of another trypanosome (11) and of a bodonid (15).

The only mitochondrial-nucleus association approaching this in other cells is that described in the ameba, *Chaos chaos*, by Brandt and Pappas (16) who discuss it in terms of mitochondrial formation by the nucleus. Numerous photographs taken of the kinetonucleus-mitochondrial structure in *T. mega* incline us to a similar interpretation: continuity between kinetonucleus and mitochondria reflects an early stage in mitochondrial development; at a later stage the mitochondria separate from the kinetonucleus and lie freely in the cytoplasm. Obviously, without definitive evidence this tentative suggestion can, at best, only help plan pertinent experiments. Should new observations suggest another meaning for the kinetonucleus-mitochondrial unit another appellation consistent with this meaning would be desirable.

I am indebted to the Belgian-American Educational Foundation for a travel Fellowship, and to Prof. Jean Brachet, Prof. Alex Novikoff, and Dr. Henri Beaufay for encouragement and assistance.

ADDENDUM:

Since this paper was submitted we have learned of descriptions of similar mitochondrial-kinetoplast structures in other trypanosomes (H. Ris, personal communication; Clark and Wallace, *J. Protozool.*, 1960, **7**, 115).

REFERENCES

1. WENYON, C. M., Protozoology, London, Bailliere, Tindall and Cox, **1**, 1926.
2. GRASSÉ, P. P., Traité de Zoologie, **1**, No. 1, Paris, Masson et Cie, 1952.
3. STEINERT, G., FIRKET, H., and STEINERT, M., *Exp. Cell Research*, 1958, **15**, 632.
4. GRASSÉ, P. P., *Compt. rend. Acad. sc.*, 1956, **242**, 858.
5. GRASSÉ, P. P., and CARASSO, N., *Nature*, 1957, **179**, 31.
6. BONÉ, G. J., and STEINERT, M., *Nature*, 1956, **178**, 308.
7. PALADE, G. E., *J. Exp. Med.*, 1952, **95**, 285.
8. WATSON, M. L., *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 727.
9. WARD, R. T., *J. Histochem. and Cytochem.*, 1958, **6**, 398.
10. ANDERSON, E., SAXE, L. H., and BEAMS, H. W., *J. Parasitol.*, 1956, **42**, 11.
11. MEYER, H., DE OLIVIERA MUSACCHIO, M., and DE ANDRADE MENDONCA, I., *Parasitology*, 1958, **48**, 1.
12. RUDZINSKA, M. A., and TRAGER, W., *J. Biophysic. and Biochem. Cytol.*, 1960, **6**, 103.
13. GRASSÉ, P. P., CARASSO, N., and FAVARD, P., *Ann. Sc. Nat. Zool.*, 1956, **18**, 339.
14. FAWCETT, D. W., Developmental Cytology, (D. Rudnick, editor), New York, The Ronald Press Co., 1959.
15. PYNE, C. K., *Compt. rend. Acad. sc.*, 1960, **250**, 1912.
16. BRANDT, P. W., and PAPPAS, G. D., *J. Biophysic. and Biochem. Cytol.*, 1960, **6**, 91.