THE EXISTENCE OF A CYTOSTOME AND THE OCCURRENCE OF PINOCYTOSIS IN THE TRYPANOSOME, *TRYPANOSOMA MEGA*

M. STEINERT and A. B. NOVIKOFF. From the Department of Pathology, Albert Einstein College of Medicine, Yeshiva University, New York. Dr. Steinert's present address is Institute pour la Recherche Scientifique en Afrique Centrale, Centre de Recherche du Katanga, Elisabethville, Katanga Republic

It is well known that pathogenic trypanosomes depend upon the macromolecules of the blood for survival but the form and manner in which such molecules enter these parasitic hemoflagellates is unknown. A direct morphological approach to this question is now possible because of two recent methodological achievements. The first is the availability of adequate methods for preparing

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thin sections for study with the electron microscope (see (1)). The second is the demonstration that ferritin molecules can be identified within cells, in electron micrographs, by means of their characteristic electron-opaque cores (2).

MATERIALS AND METHODS

The crithidias of exponentially growing cultures of *Trypanosoma mega* (3) were collected by low-speed centrifugation and suspended in a 5 per cent (*w/v*) solution of ferritin (horse spleen preparation, Nutritional Biochemicals Corporation, Cleveland, Ohio) dialyzed against constantly-stirred culture medium (3, 4) for 24 hours at 2–4°C.

Following 10 or 30 minutes in the ferritin medium the cells were sedimented once more and suspended in cold osmium tetroxide buffered at pH 7.4 (5). After fixation in the cold for 30 minutes the cells were rinsed in cold buffer and dehydrated by increasing concentrations of ethanol, with centrifugation used each time to sediment the cells. When they were in 95 per cent ethanol a higher centrifugal force was used to produce a firmly packed pellet. This was cut into minute fragments which were dehydrated in absolute ethanol and embedded, in the usual fashion, in a 1:5 mixture of methyl and *n*-butyl methacrylate containing 0.075 per cent uranyl nitrate (6). Sections were cut with a Servall Porter-Blum microtome and mounted on formvar-coated grids. These were examined with an RCA-, EMU-3B microscope, using 100 kv., at initial magnifications up to 23,000.

RESULTS AND DISCUSSION

Although all figures shown in this communication are of cells kept in ferritin solutions, the structures described in this paragraph are also present in cells grown in the usual culture medium. The crithidia is bounded by a plasma membrane which at high magnification is seen to be clearly double (Fig. 5) (a "unit membrane" of Robertson (7)). Immediately beneath the membrane are regularly spaced "pellicular fibrils" (8) (Figs. 2 to 5) which other micrographs show to be arranged longitudinally in helical fashion. In the anterior part of the cell, not far from the base of the flagellum, the fibrils change direction to form a channel (cytostome) ending deep in the cell (Figs. 2 to 5). Vacuoles of about 180 mμ in diameter may be seen within the channel (Fig. 4). They are also seen in the cytoplasm outside the channel (Figs. 3 and 5). The limiting membranes of these vacuoles have the identical "unit membrane" structure seen in the plasma membrane (Fig. 5). In Fig. 2, and to a lesser extent in Fig. 3, it is possible that the section shows the plasma membrane invaginating to form a vacuole.

The view that the vacuoles originate by the

Explanation of Figures

All figures are of *Trypanasoma mega* crithidias, fixed after 30 minutes in ferritin medium.

FIGURE 1

Oblique section, × 44,000.

Adjacent to the nucleus (*N*) is the kinetonucleus (*K*) showing a portion of its associated mitochondrion (*M*) (26). The Golgi apparatus is seen at *G*. Only the peripheral portion of the cytostome (*C*) is in the plane of section. Note that the ferritin molecules (arrow), restricted to the region of the cytostome, are not in contact with the cell membrane.

FIGURE 2

Longitudinal section, × 57,000.

This section suggests the continuity of the fibrils of the cytostome (*C*) with the pellicular fibrils (*PF*). The invagination of the cell membrane (arrow) may be a forming pinocytotic vacuole. Note that the ferritin molecules do not appear to be in direct contact with the cell membrane. The Golgi appartus is seen at *G* and mitochondria at *M*.

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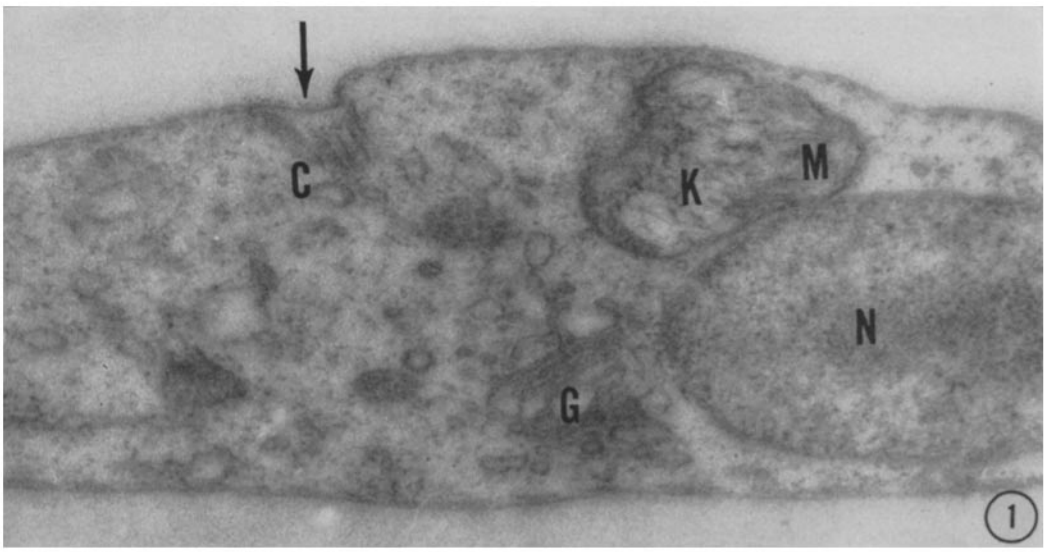

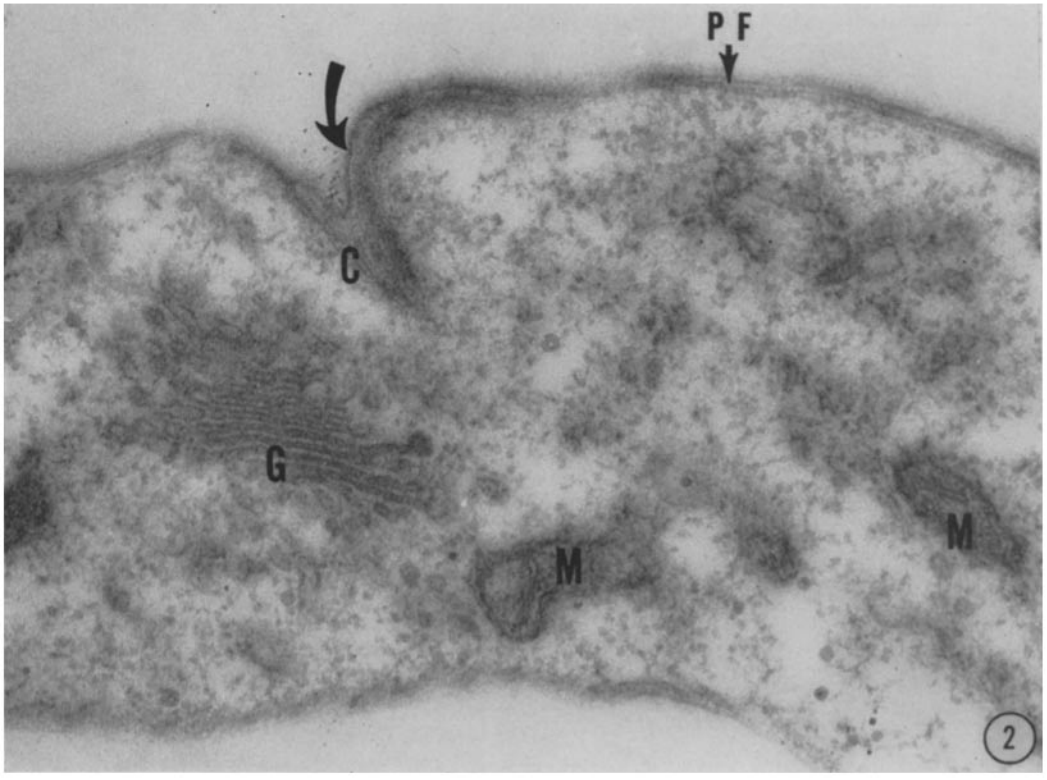

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process of pinocytosis gains strong support from the observed distribution of ferritin molecules in cells kept in ferritin medium. Ferritin molecules are found in only one area of the trypanosome surface: in the mouth of the channel (Figs. 1, 2, 3, and 5). They are also seen in the vacuoles (Figs. 4 and 5). It should be noted that the ferritin molecules are always separated from the plasma membrane and vacuole membrane by a distance of 100 A or more, representing presumably a layer of material with low electron opacity (Figs. 2, 3, and 5).

The disposition of vacuoles always in linear arrays in certain regions of the cell suggests that the vacuoles follow a definite course to the posterior end of the cell. There they apparently flow into inclusion bodies (Fig. 6) which have the appearance of lysosomes (9). In the cells kept in ferritin solution for 10 minutes the inclusion bodies already contain a great many ferritin molecules.

From these observations it seems reasonable to add the crithidia of *T. mega* to the rapidly growing list of cells in which electron microscopy reveals the occurrence of micropinocytosis. Micropinocytosis, as Holter (10) points out, appears to be essentially the same as pinocytosis (11) seen with light microscopy. *T. mega* also assumes its place with those cells in which the electron opacity of the cores within ferritin molecules has been used to provide evidence for the *direction of movement* of the vacuoles from static electron micrographs (12–15).

It is evident that the channel we have described is a permanent structure analogous to the cytostome of ciliates. Despite its smaller size we propose to refer to it by the same term, cytostome.

Our observation that ferritin molecules are not found over the entire cell surface but rather are restricted to the cytostome region may signify the presence in this region of specific binding sites such as considered to be present in bacteria (16), *Amebae* (10), and in cells generally (17). The finding that the ferritin molecules are separated from the plasma membrane and vacuole membrane, presumably by a material of low electron opacity, suggests that an outer "coat" covers the membrane where such binding sites occur, as has been suggested by the recent work of Brandt and Pappas (14) and Burgos (18). It is possible that the variability in number of ferritin molecules encountered even in adjacent pinocytosis vacuoles (Fig. 5) reflects qualitative or quantitative differences in binding sites of the cell surface which is incorporated into a vacuole at a given moment.

T. mega may prove of value in elucidating the relations of pinocytosis vacuoles and lysosomes (19, 20). If acid phosphatase activity can be demonstrated in the large inclusion bodies present in the posterior end of the cell this would establish them as lysosomes. Work from this laboratory suggests that in some cells, such as the giant ameba, *Chaos chaos* (21), and the parenchymatous cells of rat liver (19, 22), pinocytosis vacuoles acquire high levels of acid phosphatase activity immediately upon formation or very shortly thereafter, whereas in other cells, *e.g.*, those in the proximal convolutions of the rat kidney tubules, they do so only when they have traversed much of the

FIGURE 3

Transverse section, × 61,000.

A few ferritin molecules are seen (arrow) at some distance from the cell membrane. Note the fibrils of the cytostome (*C*) and the more or less transversely-sectioned pellicular fibrils (*PF*). A mitochondrion is seen at *M* and the flagellum at *F*.

FIGURE 4

Transverse section, × 57,000.

The pellicular fibrils (*PF*) are cut transversely and obliquely. Within the cytostome (*C*), cut obliquely, is a ferritin-filled vacuole (*V*); because of the plane of sectioning the vacuole membrane is not seen at the lower end. Note the similarity in structure of the pellicular fibrils and the fibrils of the cytostome (*C*).

Visible in the section is endoplasmic reticulum (*ER*), mitochondria, and an oblique section of the flagellum (*F*).

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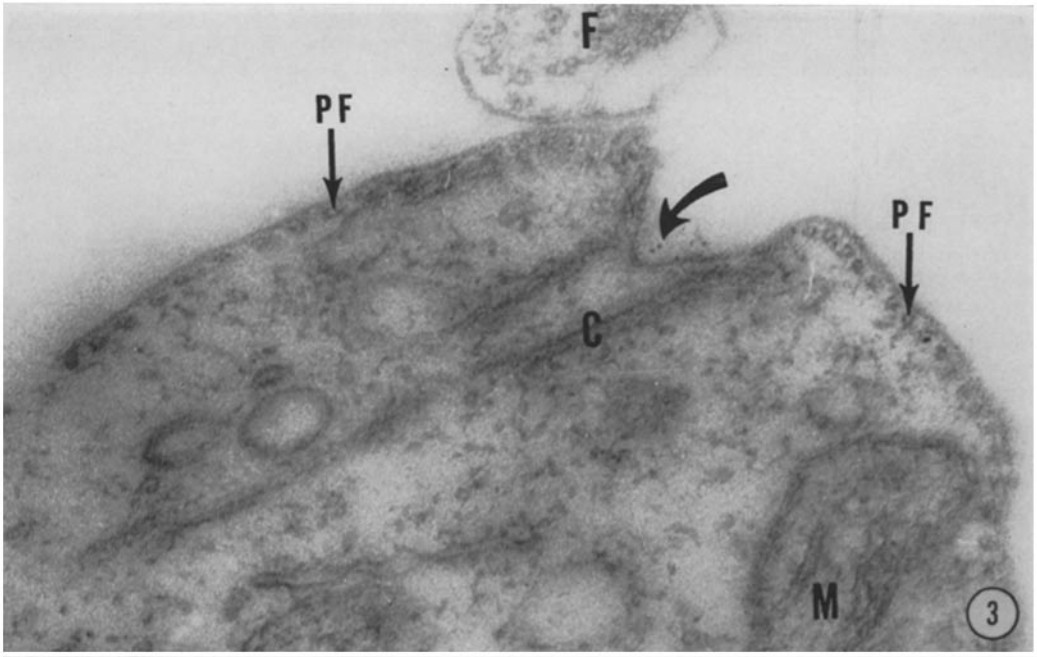

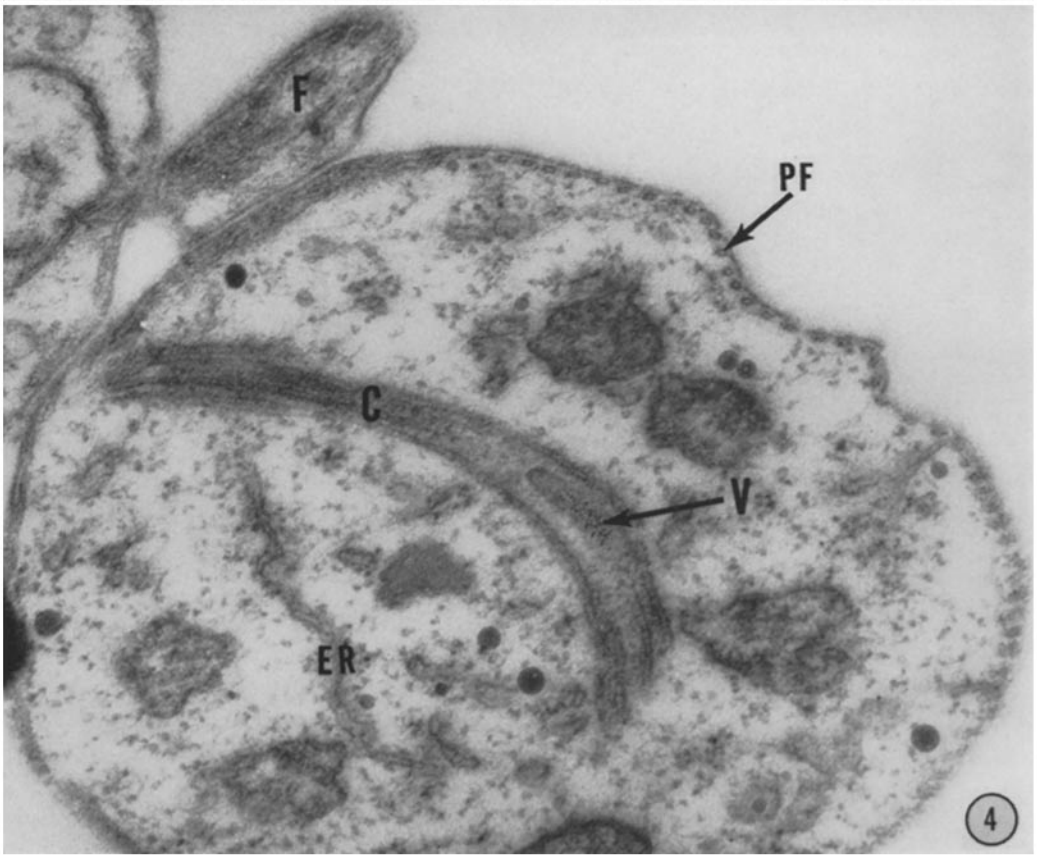

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cell (19, 23). It may be that in *T. mega* the micropinocytosis vacuoles fuse with the inclusion bodies in the same manner as such vacuoles appear to do with larger vacuoles in other cells: *e.g.*, with phagocytic vacuoles in rat erythrophagocytes (24) and pinocytosis vacuoles in cells of the proximal convolution (18, 23) and glomerular epithelium (13).

That ferritin may enter the trypanosome as intact molecules in which the cores are still recognizable indicates that macromolecules may enter the organism without first being degraded. Our observations do not establish the quantitative extent to which this occurs (see (25)). Nor do they bear on the issue of diffusion of molecules directly across the cell membrane. We can state only that we have seen no evidence that ferritin molecules cross the cell membrane directly.

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FIGURE 5

Enlargement of cytostome region, × 71,000.

At *P* the "unit" plasma membrane, with its three strata, is visible; at *V* the identical structure of the vacuole membrane is seen. Within the vacuoles variable numbers of ferritin molecules are present. Note the linear arrangement of the vacuoles.

FIGURE 6

Posterior portion, × 71,000.

The three strata of the "unit" membranes surrounding the inclusion bodies are visible at the arrow. In addition to the electron-opaque material (*OM*), also seen in inclusion bodies of cells from the usual medium, these inclusion bodies contain many ferritin molecules.

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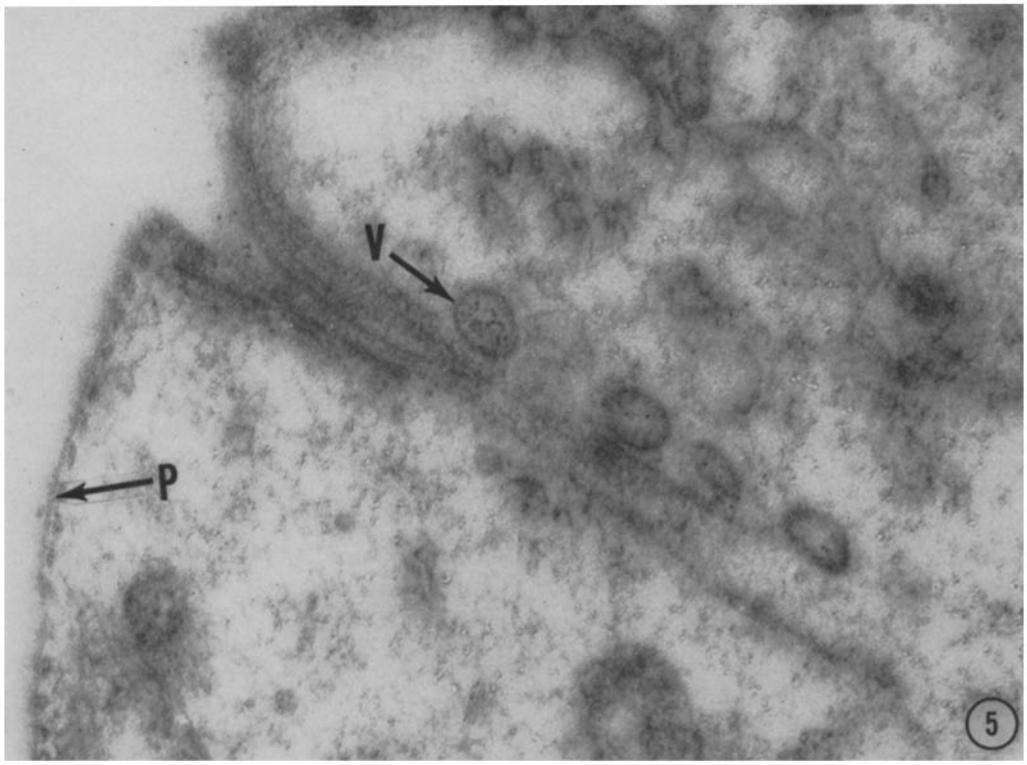

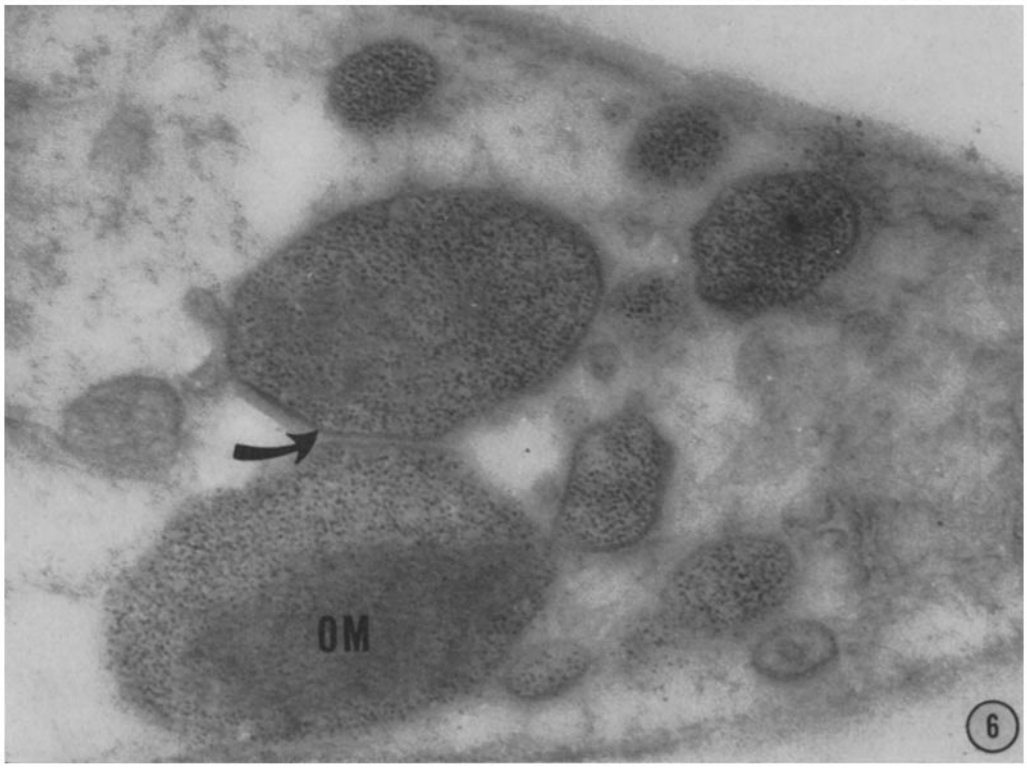

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