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Miscellanea

Ultrastructure of the Peritrophic Membrane Formation in *Glossina* Wiedemann[1, 2]

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Abstract

Study of the structure and formation of the peritrophic membrane has revealed the presence of three cell types in the annular pad of mid-gut epithelium on the basis of ultrastructure and function. Type I and II cells synthesize the precursors, possibly mucopolysaccharides, of the electron-dense first layer of the "membrane"; Type II cells, in addition, release these precursors by exocytosis. The cells of Type III are concerned with the synthesis and subsequent release of the precursors of the electron-opaque, proteinaceous second layer. The peritrophic "membrane" is thus a bilaminated structure.

The structure of this "membrane" has been discussed from the point of view of trypanosome migration. It is suggested that the trypanosomes cross this barrier in the region of the Type II cells where the first layer is in an unpolymerised granular form.

Introduction

Whether the trypanosomes (*T. congolense* and *T. brucei* subgroup) enter the ecto-peritrophic space during the very early course of the infective blood meal (Freeman, 1970) or by the classical long route of passing round the posterior free end of the peritrophic "membrane" (Yorke et al., 1933), they must at some stage cross this membrane barrier for their eventual passage to the anterior station to continue the normal developmental cycle. The study of the structure and formation of the peritrophic "membrane" is therefore a significant prerequisite to the understanding of the trypanosome migration.

The histology of the peritrophic "membrane" formation in *Glossina* was first described by Wigglesworth (1929). The present paper is concerned with the structure of the proventricular epithelial cells with special reference to the formation of the peritrophic "membrane" and its chemical nature.

Materials and Methods

The flies used were the non-teneral *G. morsitans* and *G. pallidipes* of variable age. The fly maintenance conditions have already been described (Moloo & Kutuza, 1970).

Light Microscopy: The dismembered flies were immediately immersed in Carnoy's fluid, the abdomens cut off to enhance penetration and the fixation allowed to continue for 4 hours. The proventriculus with its associated ducts was dissected out from each fly, gradually dehydrated in ethanol and embedded in

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paraffin wax (m.p. 60°C). Sections (6 μm) were stained in Mayer's haemalum, counterstained with aqueous eosin.

The peritrophic "membranes" were isolated from the living flies, fixed in Carnoy's fluid and stained with mercury-bromophenol blue (BONHAG, 1955), ninhydrin-Schiff (YASUMA & ITCHIKAWA, 1953) for protein, or periodic acid-Schiff (MCMANUS, 1948) for hexose containing substances.

Electron Microscopy: Isolated proventriculi from the living flies were fixed in 2.5% glutaraldehyde (2 h) at 4°C, washed overnight in 5% saccharose at 4°C, and then post-fixed in 2% osmium tetroxide (2 h) at 4°C. The fixatives and the washing solution were buffered to pH 7.2 with cacodylate. The tissues were prestained in 1% uranyl acetate in 70% acetone (1 h), dehydrated in acetone, and embedded in Epon according to LUFT (1960). Ultrathin sections were cut on a Reichert OmU_2 Ultrotome and mounted on Parlodion-carbon-covered grids. Sections were stained with REYNOLDS' (1963) lead citrate for 5 minutes under nitrogen gas and examined with a Philips EM 300 electron microscope.

Results

The general histological structure of the proventriculus with its annular pad of epithelial mid-gut cells and invaginated portion of the fore-gut is well shown in Figure 1. The sites of the three cell types described below are indicated.

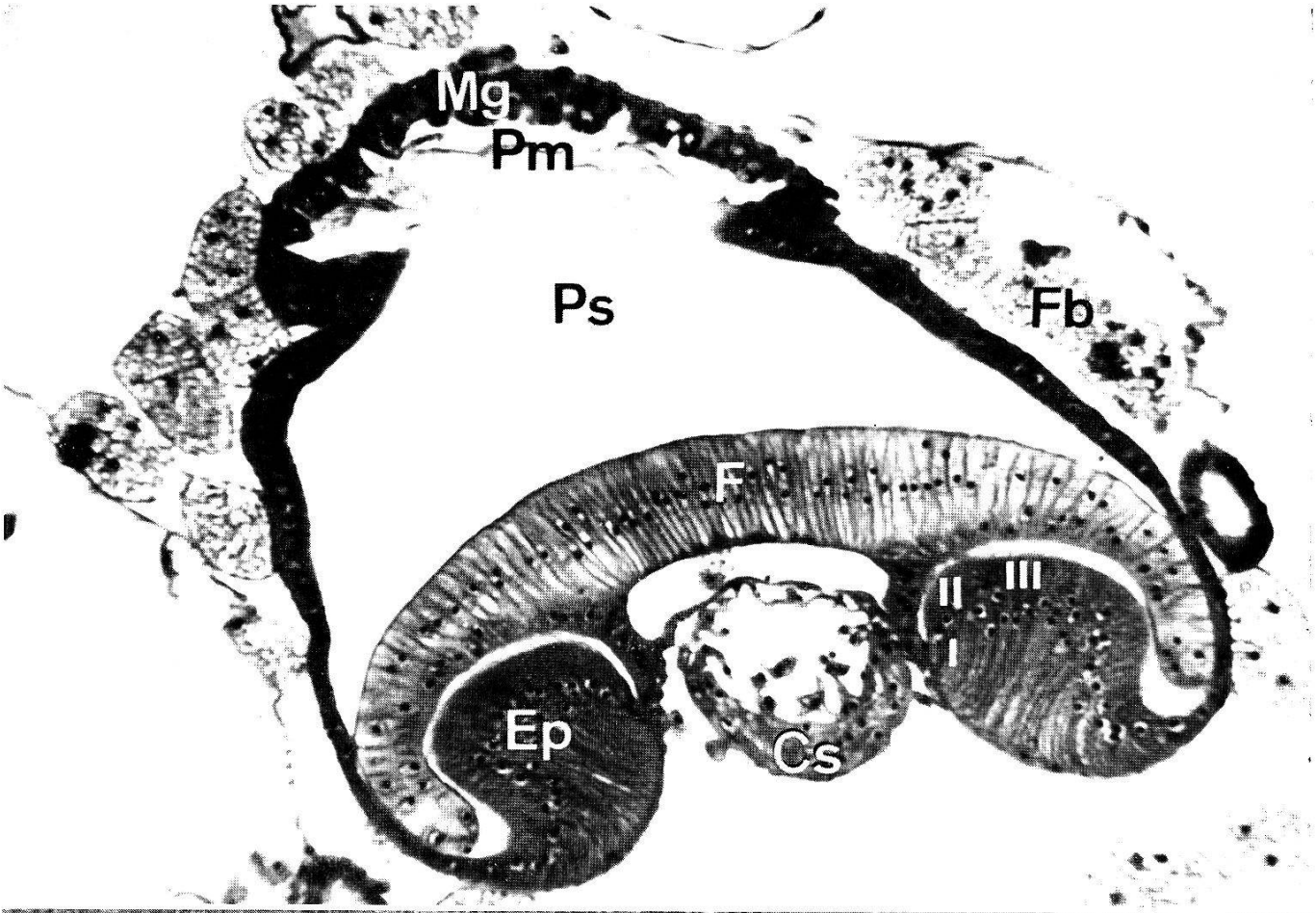
With mercury-bromophenol blue, the "membrane" obtained a clear blue colour possibly showing the presence of protein. A positive reaction for protein was obtained also with ninhydrin-Schiff. When periodic acid-Schiff staining was carried out, the peritrophic membrane gave a purplish red colouration indicating the presence of hexose substances.

Electron microscopical examination of the secretory epithelium reveals the presence of three cell types. These are distinguished by differences in their cytoplasmic organelles and plasmic microvilli. The first cell type, Type I, is confluent with the cells of the invaginated fore-gut (Fig. 3). It has a fewer microvilli which are relatively small at about 0.5 μ in length. The cytoplasm of these cells contains lysosomelike structures, small but numerous mitochondria and a large number of membrane-limited agranular vesicles possibly derived from the Golgi system.

The second cell type, Type II, is in close proximity to the fore-gut cells (Fig. 4). These cells differ from the previous type in that the microvilli are longer at about 1 μ in length and relatively more numerous. Furthermore, there are present innumerable exocytosed secretion granules in the vicinity of the microvilli. These granules are the precursors of the electrondense first peritrophic "membrane" layer.

The third cell type, Type III, is characterised by the presence in their cytoplasm of endoplasmic reticulum forming numerous and prominent granular vesicles (Fig. 5). Free RNP particles are also present. The cytoplasm contains many mitochondria which appear less electron-dense than those in cell Types I and II above. There are present microsomes, some of which are seen to be released from the larger ER vesicles. These microsomes are predominantly located in the vicinity of the microvilli which, in this cell type, are as well developed as those in Type II cells. The third type of cells are evidently concerned with the synthesis and subsequent secretion of the second, much thicker electron-opaque layer of the peritrophic "membrane" (Fig. 6).

When its secretion is completed, the peritrophic "membrane" consists of two distinct layers: the upper, or the first-formed layer is electron-dense and 0.1 μ thick while, the second is amorphous, electron-opaque and 3–4 μ thick. After the "membrane" has been drawn through the "press" (WIGGLESWORTH, *loc. cit.*), it is only 0.35 μ thick (Fig. 2).



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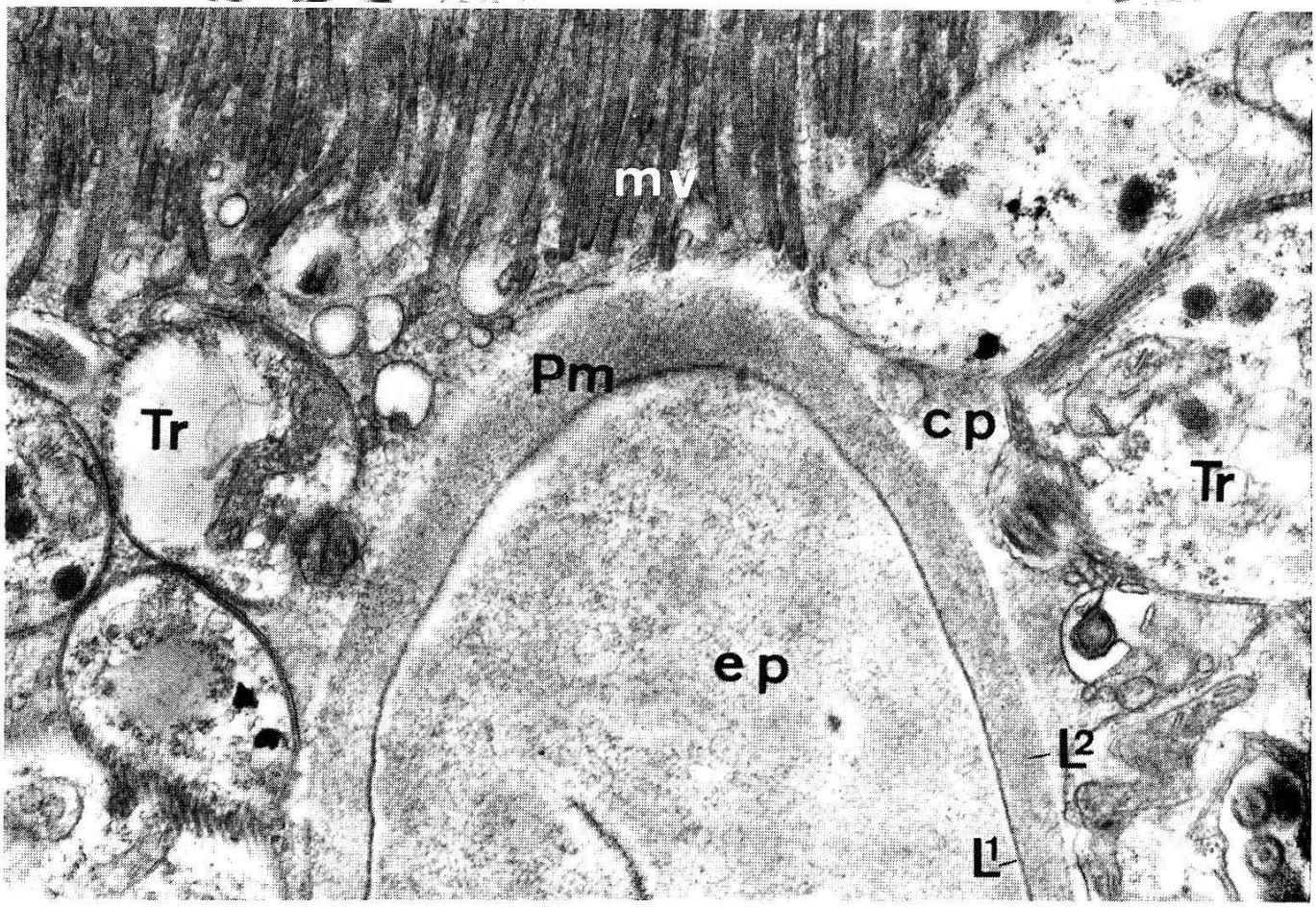

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Discussion

The present study of the peritrophic "membrane" formation in *Glossina* permits the identification of three cell types in the annular pad of mid-gut epithelium. The ultrastructural features of these cells are described above. Agranular membrane-limited large vesicles are well developed in both, Type I and II cells. These vesicles are probably derived from the Golgi complex and are characteristic of the secretory cells. The presence of the electron-dense secretion granules in the vicinity of the numerous and relatively long plasmic microvilli indicates that the cells of Type II are concerned not only with the synthesis of the precursors of first peritrophic "membrane" layer like the Type I cells but also with the subsequent release of these precursors by exocytosis. The Golgi-derived vesicles may be functionally analogous to the Golgi system of Vertebrate perikaryon, that is, they are concerned with the secretion of mucopolysaccharides (NEUTRA & LEBLOND, 1966). Positive reaction with periodic acid-Schiff would seem to support the suggestion that the electron-dense layer consists at least partly, of these sugar units.

The examination of the cytological details of the Type III cells reveals the presence of prominant and rough ER vesicles, free RNP particles and numerous microsomes. The last-named organelles are predominantly located in the vicinity of the well developed system of microvilli. It would therefore seem that these cells are concerned with the synthesis and subsequent release, by exocytosis, of the precursors of the electron-opaque second layer of the "membrane". As suggested by PALADE (1956), these ER vesicles are sites of protein synthesis. The proteinaceous nature of this layer is also indicated by the positive reaction obtained with mercury-bromophenol blue and ninhydrin-Schiff.

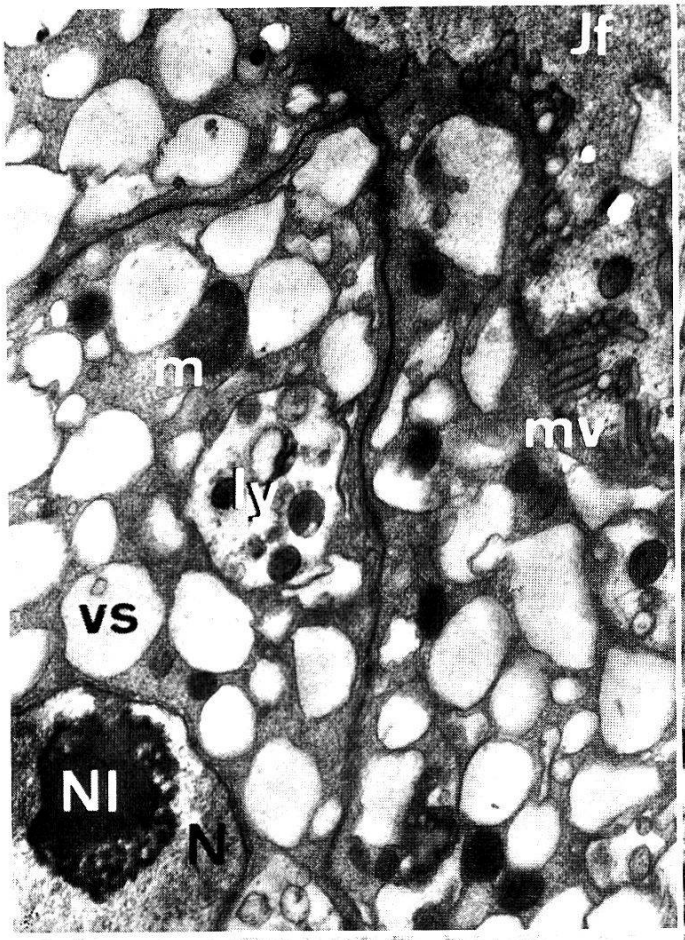
After its formation, the bilaminated peritrophic "membrane" becomes compressed to 0.33 μ as it is drawn through a narrow cleft between the proventricular wall and the invaginated fore-gut having chitinous lining. Thus, in the mid-gut the electron-dense first layer is 0.02 μ while the second-formed one is only 0.31 μ in thickness. The peritrophic "membrane" is produced very rapidly during the process of feeding and is pushed posteriorly by the tension of the advancing blood (WIGGLESWORTH, 1929). In a teneral fly, the "membrane" forms an unbroken sac and extends only upto a short length of the mid-gut (WILLET, 1966). If a teneral fly is allowed to starve, the formation of the "membrane" continues and eventually extends throughout the length of the mid-gut, although its rate of secretion is markedly low (Prof. R. GEIGY, personal communication).

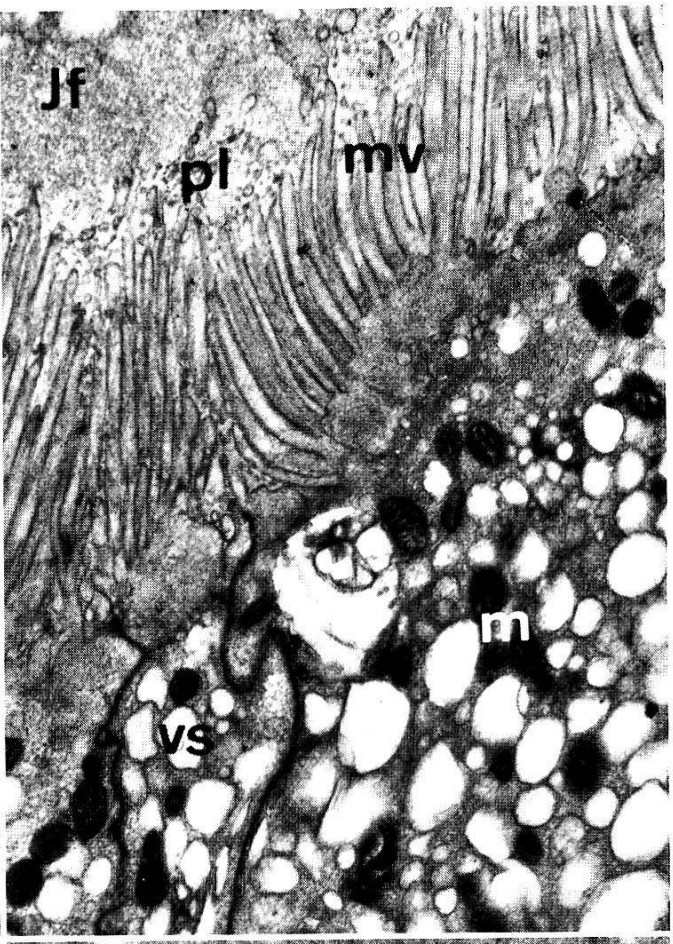
The present study also provides some indication of the possible route taken by the trypanosomes to cross the "membrane" barrier. According to FREEMAN'S (1970) suggestion, the trypanosomes penetrate the membrane not long after the ingestion of an infective blood meal. The peritrophic "membrane" is a bilaminate

Fig. 1. Transverse section of the proventriculus showing general arrangement of parts. Note the sites of cell Types I–III in the annular pad of mid-gut epithelium. 400 ×.

Fig. 2. Electron micrograph of the bilaminate peritrophic 'membrane' in the mid-gut. 18,000 ×.

Abbreviations – cl: chitinous lining; cp: ecto-peritrophic space; Cs: crop-duct sphincter; Ep: annular pad of mid-gut epithelium; ep: endo-peritrophic space; er: endoplasmic reticular vesicles; F: fore-gut invagination; Fb: fat body; Jf: fore-gut junction with mid-gut; L^1: first layer of the peritrophic 'membrane'; L^2: second layer of the peritrophic 'membrane'; ly: lysosome; Mg: mid-gut; m: mitochondria; ms: microsomes; mv: microvilli; N: nucleus; Nl: nucleolus; pl: precursors of the first peritrophic 'membrane' layer; Pm: peritrophic 'membrane'; Ps: proventricular sinus; r: free RNP, particles or ribosomes; Tr: trypanosome; vs: agranular membrane-limited vesicles.





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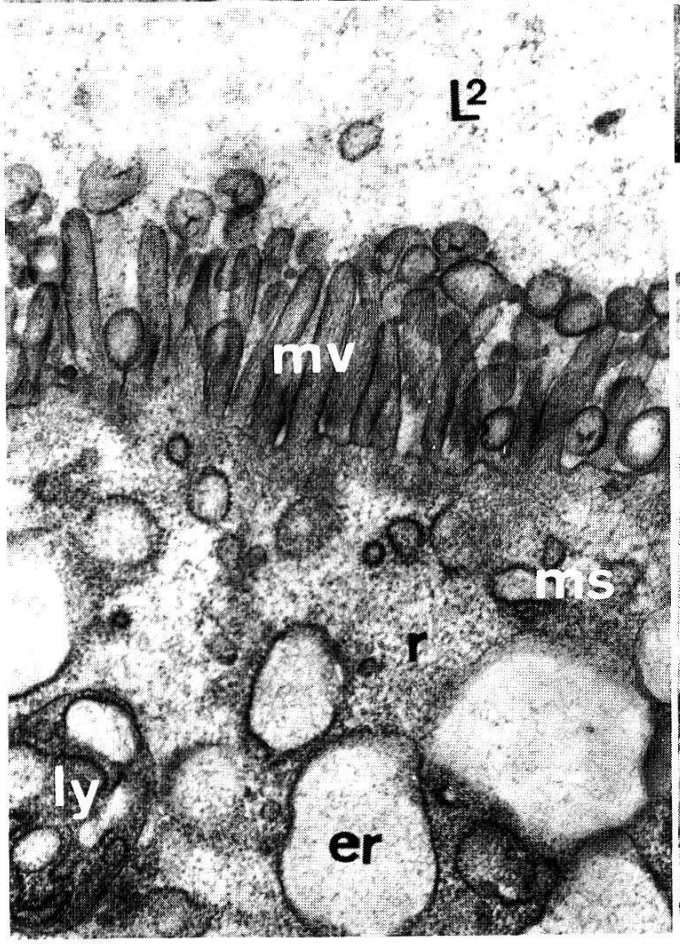

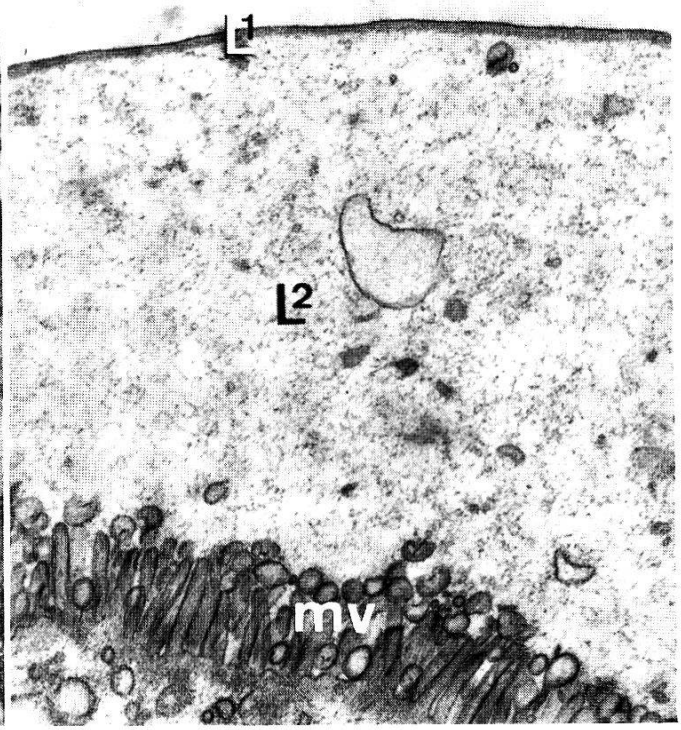

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and continuous structure. Unless it is shown that the trypanosomes have the necessary enzymatic means of digesting through this "membrane", it is difficult to conceive how they can force their way through this barrier. It is possible that a single trypanosome observed embedded in the peritrophic "membrane" (FREEMAN, *loc. cit.*) was, in fact, located in the "membrane" fold. STEIGER (unpublished observation), working with *T. brucei,* has frequently observed these trypanosomes in such folds using EM technique.

YORKE et al. *(loc. cit.)* and FAIRBAIRN (1958) suggest that trypanosomes penetrate the newly secreted fluid part of the peritrophic "membrane". This concept may be only partly correct since, although the second layer of the "membrane" is semifluid when newly secreted, the electron-dense first-formed layer is by then already partly polymerised (Fig. 6). It is very likely that the trypanosomes cross the membrane barrier in the region of the Type II cells where the first layer is still in an unpolymerised granular form.

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Fig. 3. Type I cells showing relatively few and small microvilli, and numerous membrane-limited large agranular vesicles. 10,000 ×.

Fig. 4. Type II cells; note the well developed system of microvilli and granular precursors of the first peritrophic 'membrane' layer. 10,000 ×.

Fig. 5. Type III cell; note the highly developed system of large ER vesicles and a part of the second peritrophic 'membrane' layer. 22,000 ×.

Fig. 6. Newly secreted peritrophic 'membrane'; note the relative size of the two layers. 11,000 ×.