Miscellanea : Some aspects of the surface coat formation in "Trypanosoma brucei"

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Some Aspects of the Surface Coat Formation in *Trypanosoma brucei*[1]

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Abstract

In the present preliminary study more evidence for an endogenous surface coat formation in *Trypanosoma brucei* is given.

Metacyclic salivary gland forms show numerous most probably Golgi-derived vesicles charged with coat precursor material situated close by the flagellar pocket and the base of the flagellum.

Differences between metacyclic and epimastigote forms are presented.

The results are discussed with reference to the latest ultrastructural findings in trypanosomes.

Introduction

It is known that the bloodstream and the metacyclic salivary gland form of *brucei*-subgroup trypanosomes possess a surface coat, which covers the cell membrane uniformly (VICKERMAN, 1969a).

VICKERMAN & LUCKINS (1969) showed by means of cytochemical reactions with ferritin-labelled antibodies that variant antigens are present in the surface coat and on the filopodium-like processes (WRIGHT et al., 1970), which may be correlated with the release of exoantigen (WEITZ, 1960) into the blood.

Only suggestions have existed so far what the mode of formation of this surface coat is concerned. VICKERMAN (1969a) assumes that the surface coat must be an endogenous formation and points out that ultrastructural characteristics of a secretory cell, especially the well developed Golgi-associated membrane-system, are present in *Trypanosoma congolense* (VICKERMAN, 1969b). In a recent report (VICKERMAN, 1971) the same author presumes a similar situation in *T. brucei.*

As a matter of fact, the contribution of the Golgi complex to the formation of the cell coat could clearly be demonstrated in amoebae (WISE & FLICKINGER, 1970) through the localization of polysaccharides on thin sections using the PA-silver methenamine staining method by RAMBOURG (1967). Evidence is also given by ITO & REVEL (1966) that the glycocalyx material on the microvilli of the cat intestine is derived from the Golgi area.

WETZEL et al. (1966) and BONNEVILLE & WEINSTOCK (1970) showed that the transport of coat material from the Golgi apparatus to the cell surface in the mouse colon and in the intestinal cells of *Xenopus,* respectively, is effected by vesicles.

In the following preliminary study, which is a part of more elaborate and detailed work (STEIGER, in preparation), we want to investigate and elucidate some aspects of the surface coat formation in metacyclic forms of *T. brucei* in the salivary glands of *Glossina* as revealed by electron microscopy.

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Material and Methods

Salivary glands of *Glossina morsitans* and *Glossina fuscipes* heavily infected with *T. brucei* were collected in the course of infection experiments with tsetse flies in Tororo (Uganda) (GEIGY et al., 1971).

The specimens were fixed for electron microscopy in 0.1 M cacodylate buffered (pH 7.2) glutaraldehyde, concentrations ranging from 1.5 up to 5%, for two hours at 4°C.

Washing was performed in 0.2 M cacodylate buffer (pH 7.2) with 5% saccharose overnight at 4°C. The glands were then post-fixed in 0.2 M cacodylate buffered (pH 7.2) 2% osmium tetroxide for two hours at 4°C, dehydrated in acetone, penetrated in a 1:1 mixture of propyleneoxide/Epon and embedded in Epon according to LUFT (1960).

Ultrathin sections were cut on Reichert OmU_2 and LKB Ultrotome III microtomes with glass and diamond knives, respectively, and mounted on Parlodion-carbon-covered grids.

Sections were stained with 5% aqueous uranyl acetate for 10 minutes and REYNOLDS' (1963) lead citrate for 2–6 minutes. Finally they were examined with a Philips EM 300 electron microscope.

Results

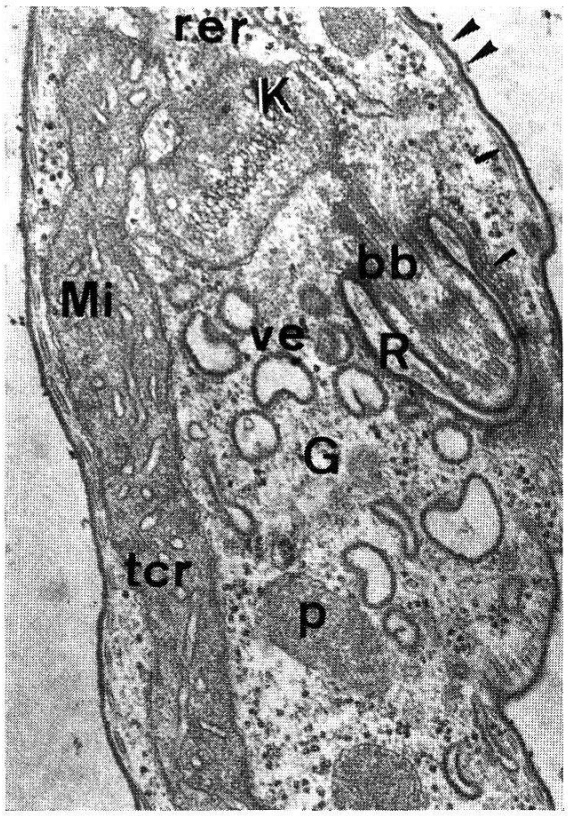
The metacyclic salivary gland form exhibits a characteristic morphology (Fig. 1). The entire cell membrane is covered with an electron-dense uniform surface coat (Fig. 1, 3). A single large mitochondrial tube, consisting of a long anterior and a short posterior part, with an electron-dense mitochondrial matrix and lined with many well developed tubular cristae, originates in the kinetoplast (Fig. 1) and extends along the cell underneath the pellicula. The pellicular microtubuli are clearly visible (Fig. 1, 3).

Moreover, the flagellar pocket (= reservoir), the base of the flagellum (= basal body), accompanied by 4 subpellicular microtubuli, some small pieces of granular ER, free ribosomes, the peroxisome-like organelles (VICKERMAN, 1969a) and a great number of vesicles can be seen (Fig. 1).

These vesicles are bounded by a unit membrane and vary considerably in size (Fig. 3). Their origin can be traced in the Golgi region (Fig. 4). There small vesicles, measuring 30–50 μ in diameter, seem to coalesce to bigger vesicles of different size (diameter: 0.1–0.3 μ). The bulk of these vesicles is concentrated in the Golgi area close to the flagellar pocket (= reservoir) (Fig. 1, 3).

Abbreviations: bb = basal body of the flagellum; cv = pinocytotic coated vesicles; F = flagellum; fr = free ribosomes; G = Golgi zone; K = kinetoplast; Mi = mitochondrium; N = nucleus; p = peroxisome-like organelles; R = reservoir or flagellar pocket; rer = rough endoplasmic reticulum; tcr = tubular cristae; ve = Golgi-derived vesicles; ≷ = coat material on the surface or in the vesicles; ⇉ = cell membrane or limiting membrane of the vesicle; ▬ = pellicular and subpellicular microtubules; ▶▶ = small electron-dense Golgi vesicles; ≈ = Golgi vesicles in fusion; ▷ = spiny coat of the coated vesicles.

Fig. 1. General view of a metacyclic salivary gland form. Note the surface coat on the cell membrane. The large mitochondrial tube and the Golgi-derived vesicles are characteristic. 29,600 ×.



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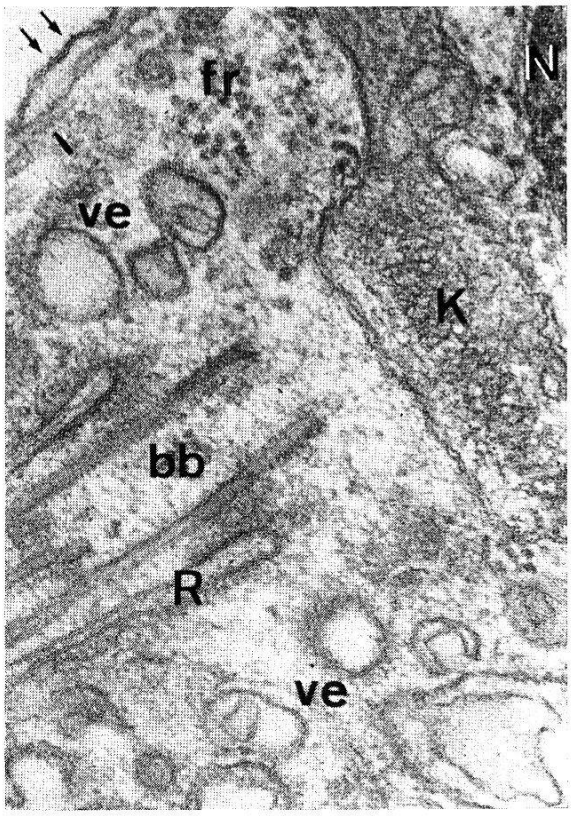

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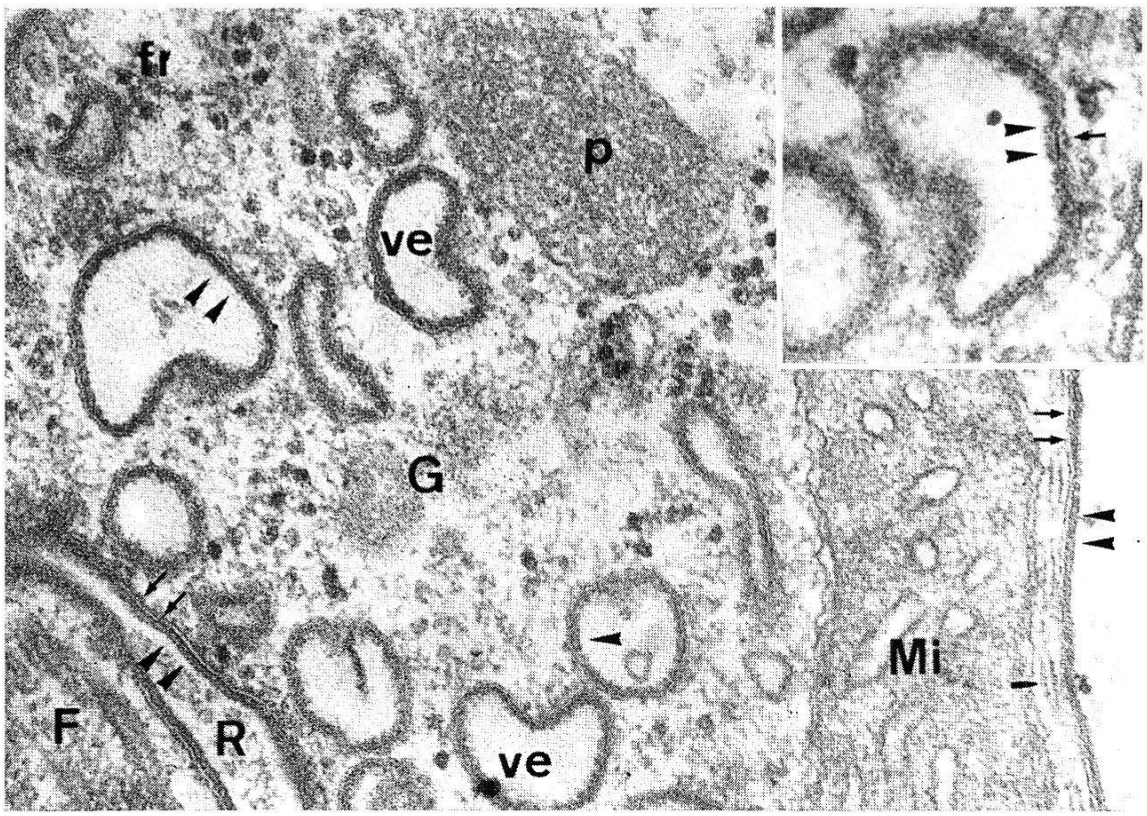

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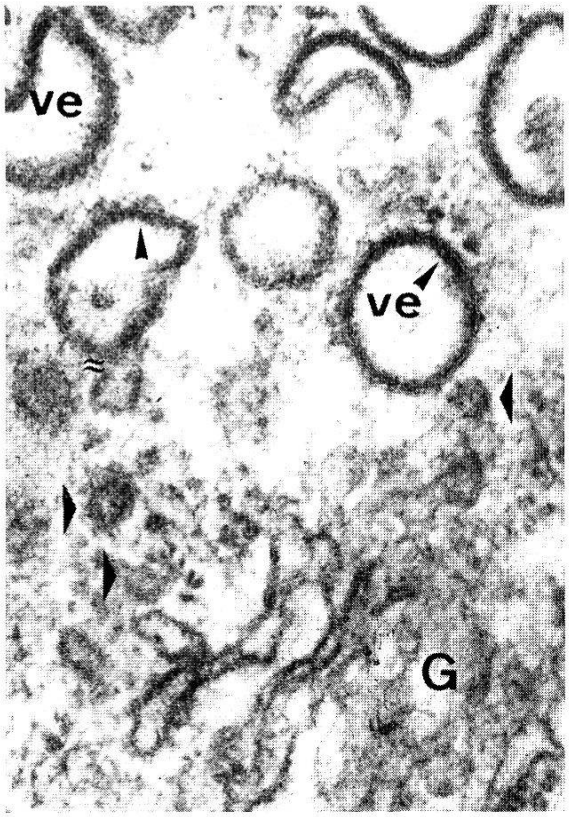
Fig. 2. Details from the cytoplasm of an epimastigote form: the base of the flagellum, the reservoir and the kinetoplast are visible. The vesicles lack the intravesicular lining typical for the metacyclic form (see Fig. 3). 57,000 ×.

Fig. 3. Details from the cytoplasm of a metacyclic form: the electron-dense intravesicular lining and the surface coat can be recognized. 70,000 ×. The inset shows the details of a Golgi-derived vesicle from a metacyclic form. Note the intravesicular localization of the coat precursor material and the limiting membrane. 113,000 ×.



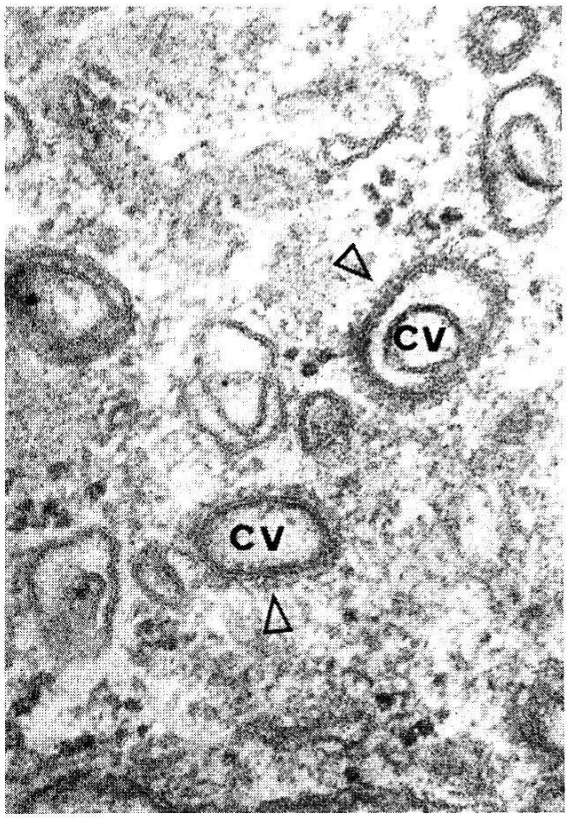


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Fig. 4. Details from a metacyclic salivary gland form: the vesicles originate in the Golgi region. Smaller vesicles seem to fuse to larger ones. 74,500 ×.

Fig. 5. Details from the cytoplasm of an epimastigote form: coated vesicles are visible. The vesicles do not possess the electron-dense intra-vesicular peripheral covering, which is characteristic for the metacyclic form. 74,500 ×.

The inner surface of the larger vesicles' membrane is covered with an electron-dense filamentous coat, which corresponds in thickness (110–130 Å) and morphology (electron-density) to the surface coat of the trypanosome (Fig. 3).

Compared to the trypomastigote metacyclic form the epimastigote form reveals distinct morphological differences. The surface coat characteristic for the infective metacyclic form is absent (Fig. 2). Vesicles situated in the Golgi zone and close to the flagellar pocket are quite numerous, but they lack the electron-dense intra-vesicular lining (Fig. 2, 5).

Besides, pinocytotic vesicles can be recognized (Fig. 5). They are quite different from Golgi-derived vesicles in having the structural specifications of coated vesicles, i.e. floccular processes on the limiting membrane (Fig. 5).

The coated vesicles are involved in the micropinocytotic uptake of proteins in insect oocytes (see e.g. ANDERSON, 1969) and were previously also described as acanthosomes in bloodstream forms of *T. congolense* by VICKERMAN (1969b).

Discussion

The results presented above give some evidence for the Golgi complex of the metacyclic form of *T. brucei* being the possible site of the synthesis of the surface coat material.

The coat material seems to be already condensed in the vesicles, which are thought to effect the transport from the Golgi region to the cell surface.

The evident concentration of these vesicles around the reservoir and the base of the flagellum leads to the view that the possible site of the coat formation,

perhaps by exocytosis of the vesicles' contents, may be the flagellar pocket. This is in accordance with VICKERMAN's (1969b) opinion. Exocytosis might be, similarly to pinocytosis (GEIGY et al., 1970), favoured by the absence of pellicular microtubules. However, it must be admitted that actual exocytosis has not been observed so far.

The strictly peripheral localization of the coat precursor material on the inner surface of the vesicles could be as well an osmotic fixation artefact. Similar peripheral lining was observed in pinocytotic vesicles using peroxidase as a protein tracer in the oogenesis of *Periplaneta americana* (ANDERSON, 1969), in the vitellogenesis of the tick *Ornithodorus moubata* (JENNI, 1971) and in bloodstream forms of *T. brucei* (GEIGY et al., 1970). The demonstration of coat precursors in the Golgi-derived vesicles of amoebae often reveals also a peripheral covering (WISE & FLICKINGER, 1970). On the other hand the possibility of specific membrane binding sites for glycoproteins can not be excluded.

The morphological features of the vesicles and sacs of secretion described by VICKERMAN (1969a, 1969b) in *T. congolense* compared to the transport vesicles in our case are quite different. Yet, in the stages of *T. brucei* observed by us, the agranular ER is insignificant or absent. Judging from his micrographs we think that VICKERMAN's (1969b) "cytoplasmic membrane system" in *T. congolense* is considerably involved in the formation of different cell organelles and that the sections prepared for the paper cited before were not thin enough to clarify the role of the Golgi region and the vesicles concerned.

In the publication by WRIGHT & HALES (1970) on the cytochemical localization of polysaccharides in the surface coat of *T. brucei,* some vesicles in the cytoplasm stained positive for polysaccharides. Though they were not examined with regard to transport problems, they could well be identical with the vesicles we have found.

Furthermore, the absence of coat precursor material from the vesicles in the epimastigote (= Crithidia) forms is another indication for the different activity of the Golgi complex in the various developmental stages and the role of the Golgi-derived vesicles in the formation of the surface coat in metacyclic forms of *T. brucei.*

Some vesicles demonstrated show invaginations of their limiting unit membranes. This might be an artefact possibly caused by slightly inadequate fixation of the cells.

It must be added that trypanosomes transforming from the epimastigote to the trypomastigote stage, as defined by the juxta-nuclear position of the kinetoplast during its mitochondrium-dependent migration from the anterior to the posterior position, do not possess any surface coat, and that the coat formation does not set in until the trypanomastigote stage has been reached. This observation modifies VICKERMAN's (1969a) suggestion that the transforming trypanosome has got a somewhat thinner coat.

In another experiment we shall try to demonstrate polysaccharides cytochemically in order to get more information on the transport mechanism.

As it was indicated before, this and further pending questions will be the subjects for a more detailed work.

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