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Nuclear Coat and Viruslike Particles in the Midgut Epithelium of *Glossina morsitans* spp.

L. JENNI and S. BÖHRINGER

Abstract

The ultrastructural aspects of the nuclear coat formation in midgut epithelial cells of pupae and adult flies of *G. morsitans* spp. are described. Out of four different species of *Glossina* examined, this peculiar structure was only found in *G. morsitans* spp.

Three different types of viruslike particles were found in midgut epithelial cells. One type which is of the same kind of particle found in the salivary glands, lies inside of cytoplasmic vesicles. Two other types of particles were detected in the nuclei.

Abbreviations used in the figures

| | |
|----------------------|-----------------------------------|
| cl = cluster | mt = microtubule |
| cr = crystal | nc = nuclear coat |
| cy = cytoplasm | nu = nucleus |
| go = Golgi apparatus | rer = rough endoplasmic reticulum |
| mi = mitochondrion | ve = vesicle |

Legends of figures. All micrographs shown were made from midgut material of *G. morsitans centralis* and *G. m. morsitans*.

Fig. 1. ♂, 78 days after emergence. Nucleus of a midgut epithelial cell (middle part of the gut) surrounded by the nuclear coat. 12,000 ×.

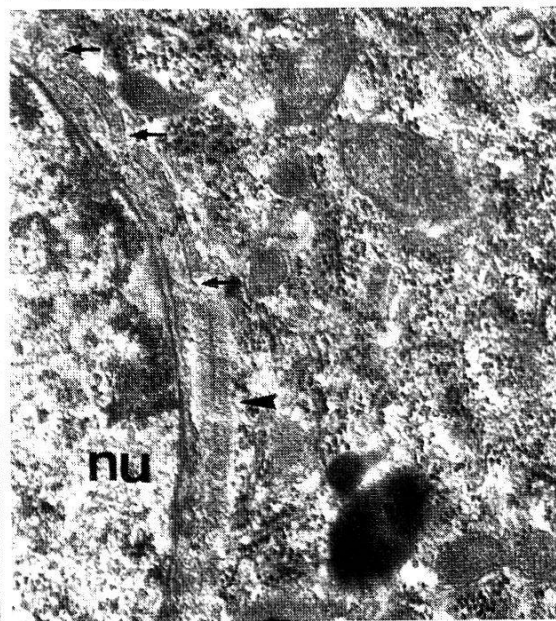
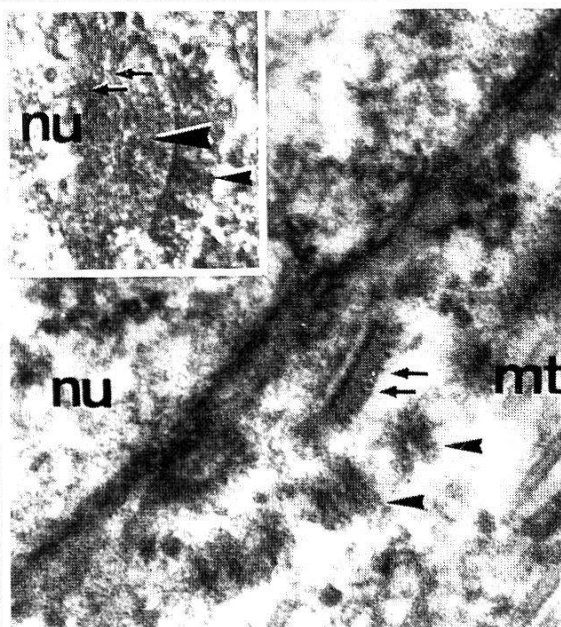
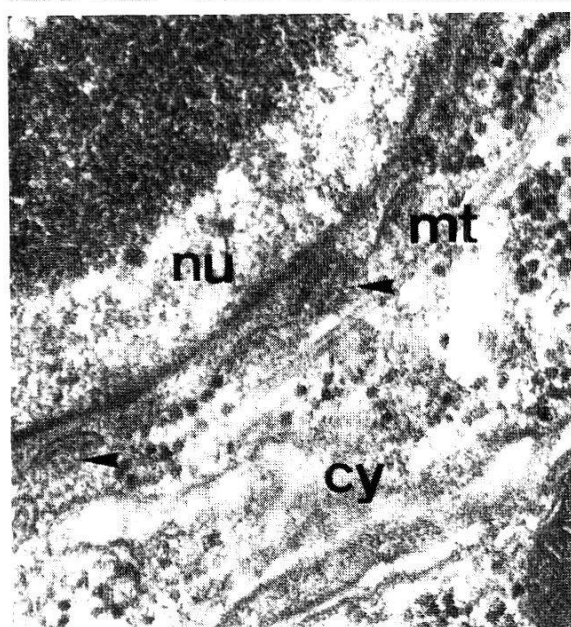
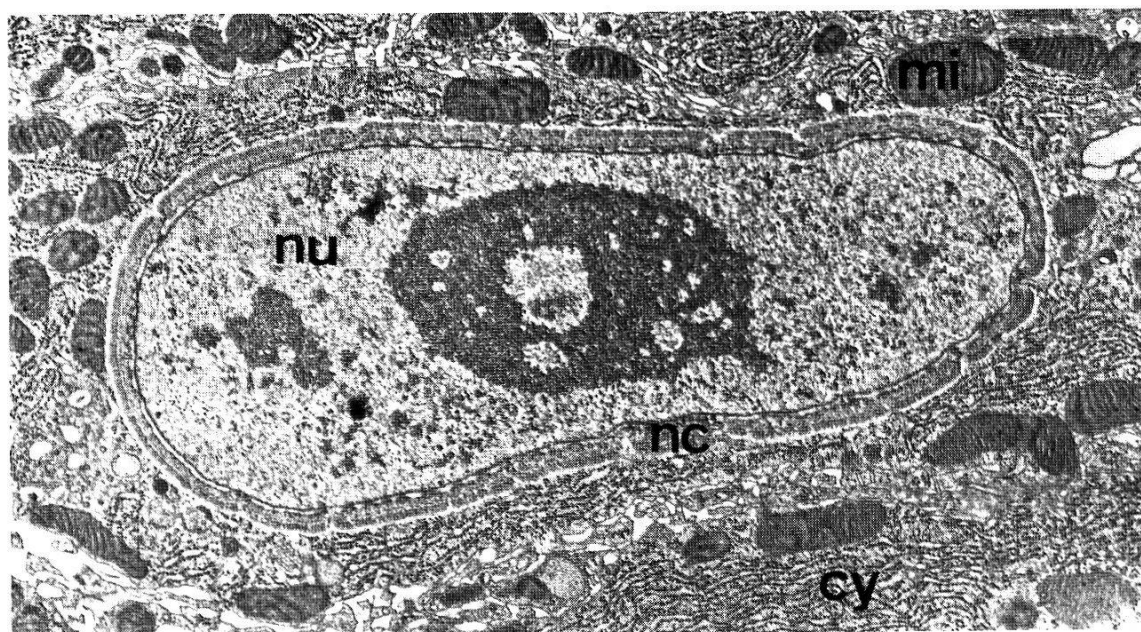
Fig. 2. ♂, pupa, 25 days old. First appearance of electron-dense material (►) at the outer membrane of the nuclear envelope. 54,000 ×.

Fig. 3. ♂, pupa, 25 days old. Rodlike structure (→) lying close by the nucleus. In addition, patches of electron-dense material occur in the cytoplasm (►). 86,000 ×.

Inset. ♀, pupa, 29 days old. The space between the rodlike structure (►) and the nuclear membranes (→) is filled with electron-dense material (►). 54,000 ×.

Fig. 4. ♂, pupa, 25 days old. Nucleus surrounded by a coat (►) formed by a rodlike structure. A possible variant (→) of this structure is seen in the vicinity of the coat. 44,200 ×.

Fig. 5. ♂, teneral fly. An other variant rodlike structure (►) surrounds this nucleus. The coat is discontinuous (→). 27,000 ×.



Introduction

Peculiar structures formed about the nuclei of different insect cells have been repeatedly reported. REINHARDT (1975, 1976) describes a nuclear halo in differentiated midgut cells of *Tunga penetrans*. Recently, BAUER (pers. communication) has found a similar halo in midgut cells of *Culex pipiens pipiens* females. Besides these findings in hematophagous insects, a nuclear coating of the oocyte nucleus of *Drosophila virilis* was shown by KINDERMANN & KING (1973).

The present paper gives evidence of a nuclear coat in midgut epithelial cells of both sexes of *Glossina morsitans* spp. In two preliminary papers (JENNI 1973, JENNI & STEIGER 1974a), viruslike particles in the salivary gland and in the midgut epithelium of *Glossina morsitans* spp. were described. These particles are distinct from those found in nuclei of midgut epithelial cells of *Glossina fuscipes fuscipes* (JENNI & STEIGER 1974b). This paper will give an additional account of the occurrence of viruslike particles in the midgut epithelium of *Glossina morsitans* spp.

Material and Methods

The following species of *Glossina* were investigated:

Glossina morsitans centralis (Machado), emerged from puparia collected at Singida, Tanzania.

G. m. morsitans (Machado), post-teneral flies caught near Ifakara, Tanzania.

G. m. morsitans (Machado), pre-emerged flies and flies emerged from puparia obtained from the laboratory colony at Langford (Bristol).

G. austeni, emerged from puparia from Langford.

G. fuscipes fuscipes (Newst. 1910), emerged from puparia, which were either collected in the field (Lugala, Lake Victoria) or obtained from a laboratory colony (EATRO) of the same origin.

G. brevipalpis, post-teneral flies caught near Ifakara.

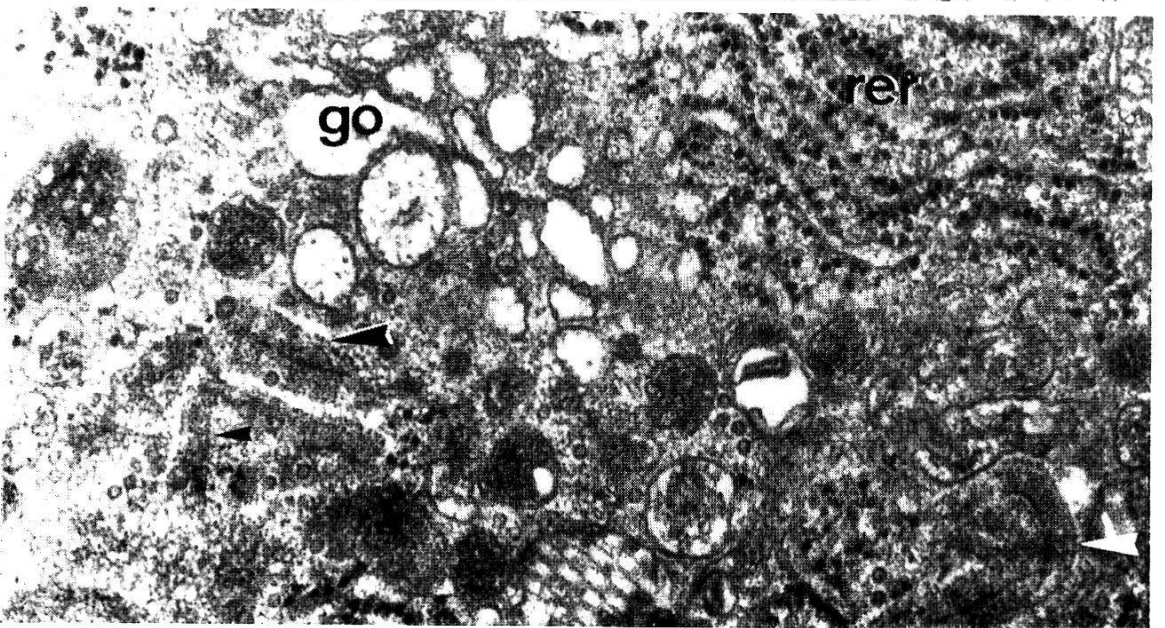
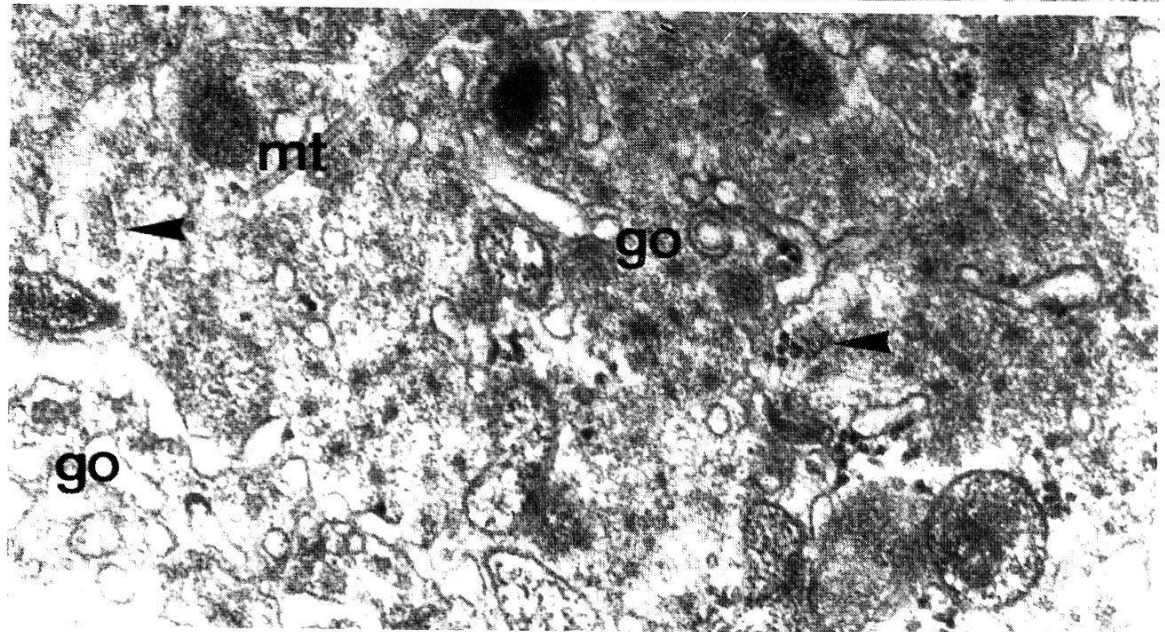
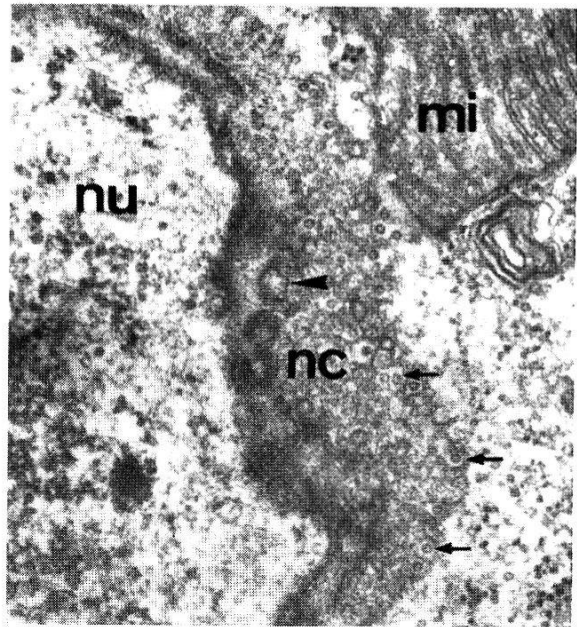
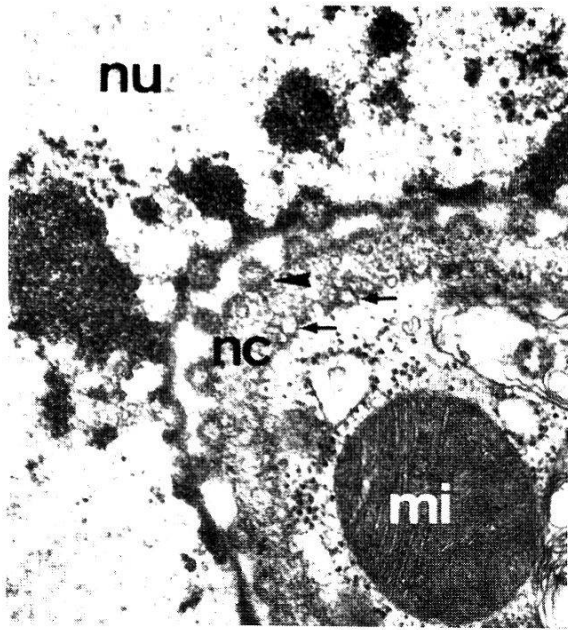
The entire midguts of adult flies (both sexes) as well as of pre-emerged flies were dissected and processed for electron microscopy according to routine methods (BÖHRINGER & HECKER 1975). Double stained (uranyl acetate/lead citrate) ultrathin sections were examined and recorded in Philips EM 300 and Zeiss EM 9 electron microscopes.

Fig. 6. ♀, pupa, 25 days old. Tangential section of the nucleus with nuclear pores (►). The nuclear coat shows a honeycomb-like structure with pores (→). 27,000 ×.

Fig. 7. ♂, 40 days after emergence. In a tangential section, beside nuclear pores (►), the pores of the honeycomb-like structure of the coat contain small vesicles (→). 36,000 ×.

Fig. 8. ♀, 77 days after emergence. Small pieces of a single rodlike structure (►) are often found in close association with the Golgi apparatus. 45,000 ×.

Fig. 9. ♂, 78 days after emergence. Beside the single rodlike structure (►), also a variant (double) structure(◼) close to the Golgi-zone does occur. 45,000 ×.



Results

From all the species of *Glossina* examined in this study, only *Glossina morsitans* spp. showed a nuclear coating in the midgut epithelial cells. This coat was found in both sexes of flies.

The first indications of specific modifications leading to the formation of the nuclear coat in midgut cells (Fig. 1) appear in pupae 25 days after larviposition (Fig. 2). At this stage, electron-dense material is found in the cytoplasm being attached to the nuclear envelope. In addition, different deposits can be recognized in the cytoplasm of some cells. These deposits consist of rodlike structures and periodic patches of dense material, orientated parallel to the nuclear envelope (Fig. 3). The space between the rod-shaped pieces and the nuclear envelope is gradually filled with "amorphous" material (Fig. 3, inset). In Fig. 4 the nuclear coating is nearly completed. Its structural arrangement is similar to Fig. 3 (inset). Additional pieces of rodlike material are seen in the cytoplasm.

The clear zone which occurs between the coat and the cytoplasm (Figs. 1, 4 and 10) may be due to the different shrinkage of nucleus/coat and cytoplasm during the fixation process.

Fig. 5 shows a nuclear coat in a teneral fly. The coating consists of another variant of rod-shaped structure with a striated median line. In tangential sections the coat shows a honeycomb-like structure (Fig. 6). In a later stage the pores of the coat contain small vesicles (Fig. 7).

Generally, the formation of the nuclear coating is very heterogenous in regarding the temporal appearance of the coat forming material as well as its initial structural character. With the exception of the so-called clear cells (BÖHRINGER in prep.) and the mycetocytes, the nuclear coating takes place in every cell of the midgut epithelium. The coating starts in cells of the posterior and middle part of the midgut. On an average, flies older than 30 days exhibit the nuclear coat in midgut cells of the anterior part also.

While in midgut cells of pupae and of teneral flies, the possible place of origin of the coat material could not be observed, small pieces of different rod-shaped types could be found in midgut cells of aged flies lying in close association with dycytosomes (Figs. 8, 9). This may indicate that the Golgi apparatus is involved in the synthesis or polymerisation of the coat forming material. But

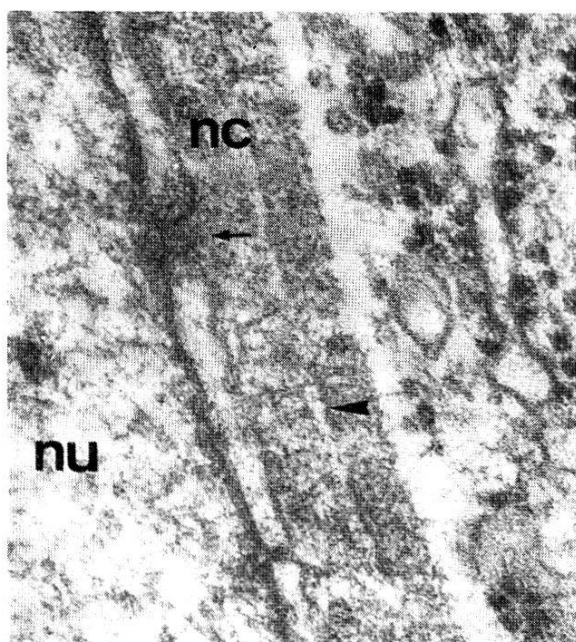
Fig. 10. ♀, 77 days after emergence. The fully formed nuclear coat and a nuclear pore (→) are shown. The coat is separated by an electron-dense line (▶) into two parts. 100,000 ×.

Fig. 11. ♂, 28 days after emergence. The outer part (▶) of the nuclear coat, which is more dense than the inner part, resembles very much the single rodlike structure (→) lying nearby in the cytoplasm. 80,000 ×.

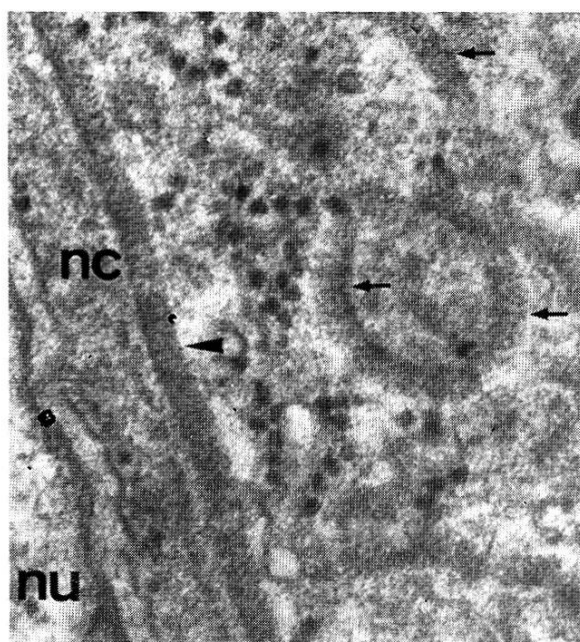
Fig. 12. ♂, 73 days after emergence. Double rodlike structure (▶▶) (as in Figs. 5 and 9) lying between mitochondrions. The double structure is equally separated by a dense line (→). 45,000 ×.

Fig. 13. ♂, 78 days after emergence. The same structure as in Fig. 12 forms (▶▶) a ring in this section. Inside the ring a possible variant of the latter structure forms a core (→▶). 45,000 ×.

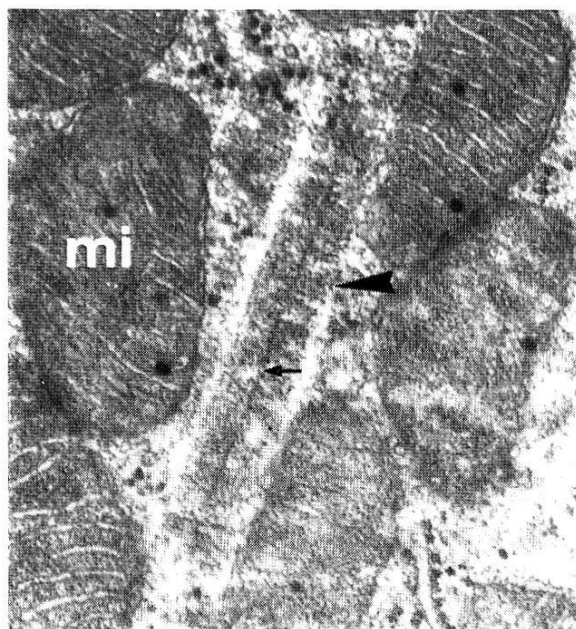
Fig. 14. ♂, 78 days after emergence. General view of multiple rodlike double structures lying in parallel rows (▶▶) in the cytoplasm. 24,000 ×.



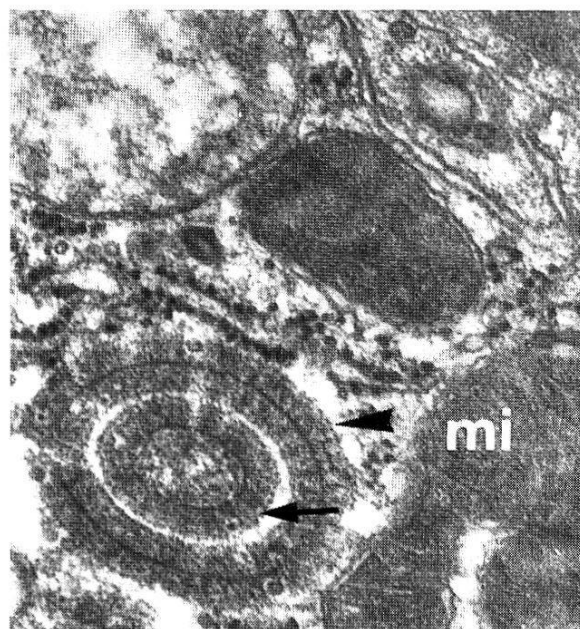
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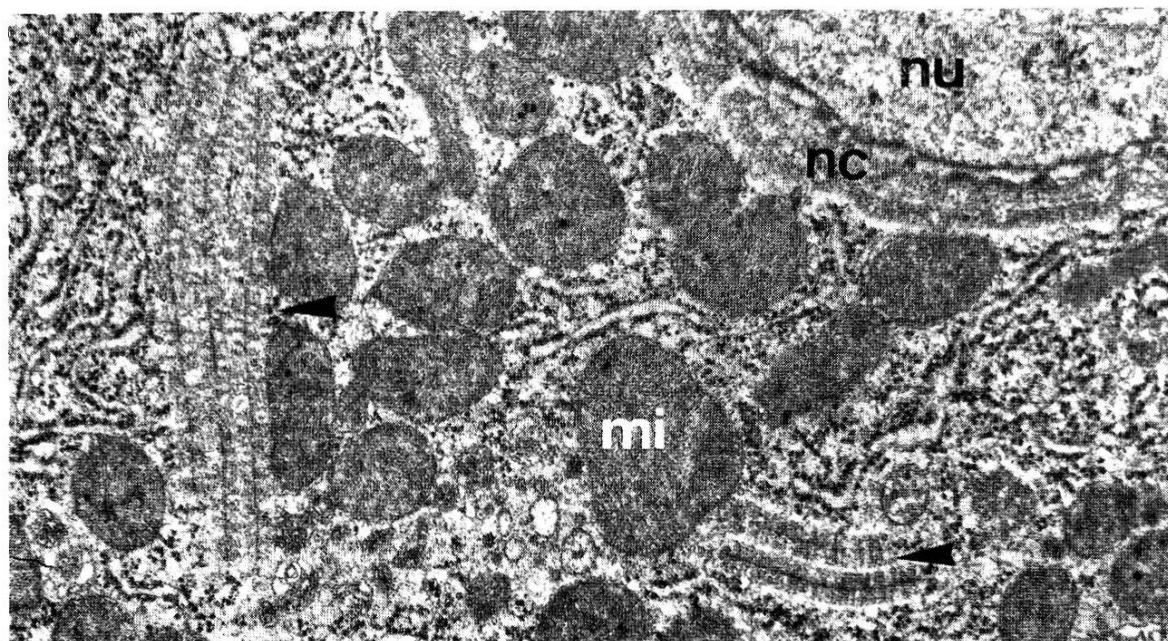
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from a static picture it is risky to determine whether the variant structures found in the vicinity of the dyctiosomes are actually entering the coating of the nuclei. Fig. 11 shows pieces of deposits in the cytoplasm near the coat. The coat reaches a thickness of about $0.12\ \mu$. The structure of the completely formed coat varies (e.g. Fig. 10 compared with Fig. 11).

In older flies an accumulation of distinct structures is often found in the cytoplasm (Figs. 12–14). Single pieces (Fig. 12) or several pieces lying together in parallel (Fig. 14) can be found. Very rarely ring-shaped bodies were found (Fig. 13).

Viruslike particles (VLP) were found in *G. f. fuscipes* and in *G. morsitans* spp. The data given here are restricted to *G. morsitans* spp. The occurrence of VLP showed no difference between both sexes of flies.

VLP were already found in cytoplasmic vesicles of midgut epithelial cells of pupae (Fig. 15). These particles resemble very much those, found in salivary glands (JENNI & STEIGER 1974a). The spherical particles are composed of a membranous envelope and an electron-dense core. The envelope measures 480–500 Å and the core 240–260 Å in diameter. In Fig. 16, one particle is seen at the periphery of a cytoplasmic vesicle either entering or leaving the vesicle. The number of particles in the vesicles increases with the age of the fly (Fig. 17). Fig. 18 shows VLP of the same kind as those in Figs. 15–17 forming a crystal in the cytoplasm. These crystals are rarely found.

Nuclei of midgut epithelial cells of older flies contain very often clusters of particles measuring 260–280 Å in diameter (Fig. 19). Serial sections were examined in order to verify the vesicular structure of these particles.

A third type of VLP was found in *G. m. centralis* in the nuclei of midgut epithelial cells (Fig. 20). These particles are rodlike and consist of an enveloped core (Fig. 20, inset). The envelope measures 320–340 Å and the core 140–160 Å in diameter.

Beside the salivary gland and the midgut epithelium, no VLP have been found so far when fat body, Malpighian tubules and reproductive organs were examined.

Fig. 15. ♂, pupa, 25 days old. VLP (►) inside a cytoplasmic vesicle in an epithelial cell of the midgut (middle part). Some of the particles are not fully surrounded by a membranous envelope (▶). Pieces of membranes (→) not associated with particles are seen. 80,000 ×.

Fig. 16. ♂, teneral fly. Vesicle with a VLP (▶). An other particle (►) is either entering or leaving the vesicle. 143,000 ×.

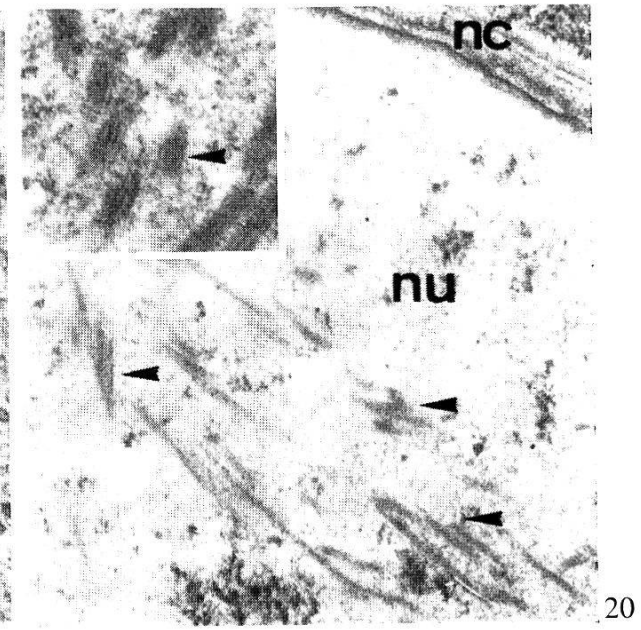
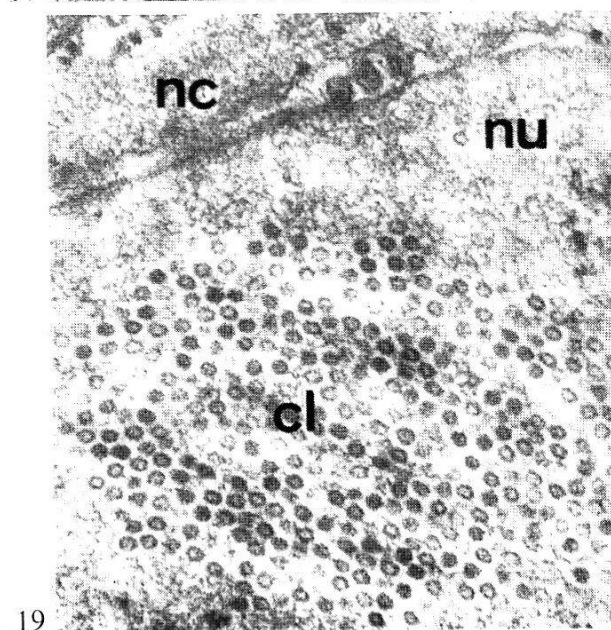
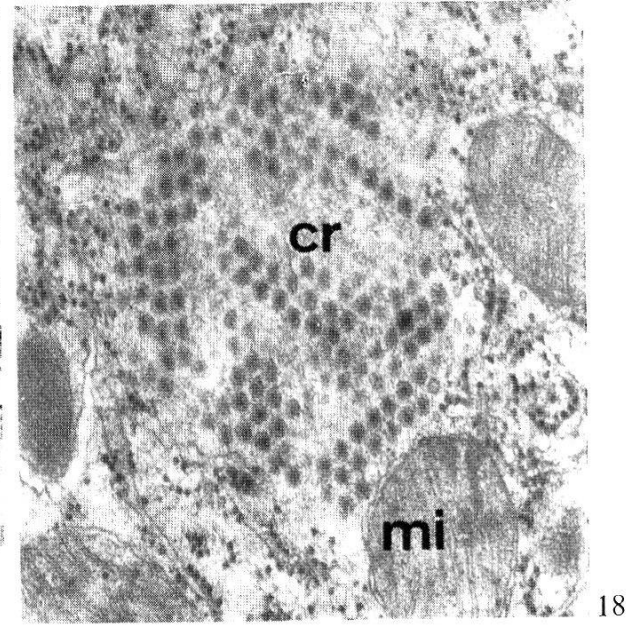
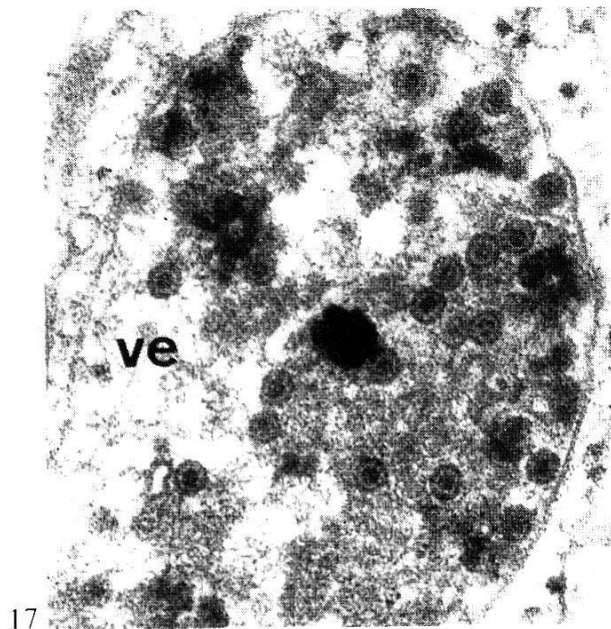
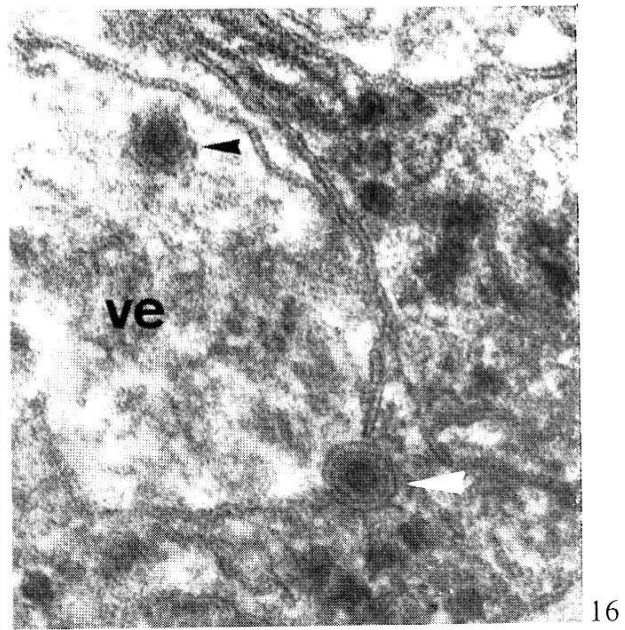
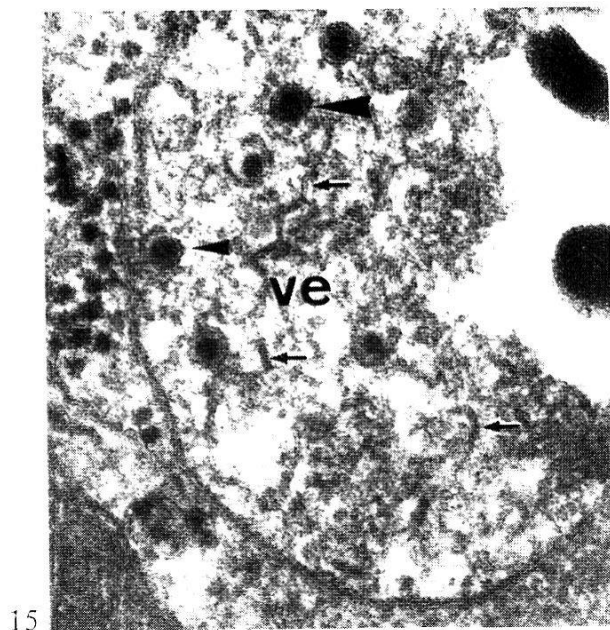
Fig. 17. ♀, 43 days after emergence. Cytoplasmic vesicle (posterior part of the midgut) contains numerous VLP. 80,000 ×.

Fig. 18. ♂, 48 days after emergence. VLP forming a crystal in the cytoplasm of a midgut epithelial cell (middle part). 36,000 ×.

Fig. 19. ♂, 78 days after emergence. Cluster formed by VLP having a different structure than those in Figs. 15–18 are often found in the nuclear plasma of midgut cells. 54,000 ×.

Fig. 20. ♂, 28 days after emergence. Rod-shaped viruslike material (▶) does occur in the nuclear plasma. 36,000 ×.

Inset: Same fly. Transverse section of the rod-shaped structure (▶) shows the enveloped core. 80,000 ×.



Discussion

From the morphological data we have so far, the functional significance of the nuclear coat is difficult to interpret. One fact worth stressing is that the coating is restricted to midgut epithelial cells. We found no nuclear coats in any other fly organ. Furthermore, this unique structure was only found in *G. morsitans* spp. and in none of the other tsetse fly species examined in this study.

REINHARDT (1975, 1976) discusses the function of the nuclear halo in relation to some additional functions of the enlarged midgut nuclei in *Tunga penetrans*, as well as the possibility that the halo may regulate the transport mechanism for RNA from the nucleus to the cytoplasm. KINDERMANN & KING (1973) assume that the nuclear coating prevents the nucleocytoplasmic exchange of high molecular weight material and thus helps to insulate the oocyte chromosomes from the influence of those compounds that stimulate transcription in the sister nurse cells. If the VLP represent true viruses, then the coat in *G. morsitans* spp. could act as a barrier to the penetration of virus compounds through the nuclear envelope. On the other hand, the coat (in its complete form) could be a general selective barrier in nucleocytoplasmic exchanges in older flies.

The substantial composition of the coat is so far unknown. There is no histochemical evidence for RNA, as was examined with the regressive staining method by BERNHARD (1969).

The VLP found in the midgut epithelial cells give a further ultrastructural account of VLP in *Glossina morsitans* spp. The comparison of these particles with arboviruses was discussed previously (JENNI & STEIGER 1974a). In contrast to the findings in the salivary glands, two further types of VLP have been detected in nuclei of midgut epithelial cells. As in midgut cells of *G. f. fuscipes* (JENNI & STEIGER 1974b), cytopathic effects could not be seen with certainty in our material.

Acknowledgments

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