OBSERVATIONS WITH THE ELECTRON MICROSCOPE OF MYXOMA VIRUS ON MOSQUITO MOUTHPARTS

By B. K. Filshie*

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Summary

The stylets of *Aedes aegypti* mosquitoes which have fed on rabbits infected with myxomatosis have been examined with the electron microscope. Virus particles were observed adhering to the mouthparts. The means of attachment, the total number of particles, and the distribution along the stylets are discussed.

I. Introduction

The transmission of myxomatosis by the mosquitoes *Aedes aegypti* and *Anopheles annulipes* has been studied extensively (Fenner, Day, and Woodroofe 1952, 1956; Day, Fenner, and Woodroofe 1956; Fenner and Ratcliffe 1965) and all results are compatible with a purely mechanical transmission. No multiplication of virus occurs within the mosquito, and virus imbibed with the blood of an infected rabbit is not involved in subsequent transfer of the disease. The piercing stylets, the maxillae, mandibles, labrum–epipharynx, and hypopharynx must pick up virus particles as they pass through infected cells of the epidermis and dermis of the diseased animal, and some proportion of these particles is wiped off the mouthparts when the mosquito again bites. Fenner, Day, and Woodroofe (1956) demonstrated that some species of mosquitoes are more efficient vectors than others and suggested the structure of the stylets may be related to the differences observed.

It has been shown (Fenner and Ratcliffe 1965) that because of the irregular distribution of virus-containing cells in the lesions of infected rabbits, great variation occurs in the load a mosquito may acquire; some mosquitoes may remain uninfective, whilst in others there may be several thousand infective particles on the mouthparts.

The present study was undertaken to obtain visual evidence of the virus particles on the mouthparts, their exact distribution, mode of attachment, and, if possible, a rough estimate of the total number of virus particles (infective plus non-infective) on the stylets.

II. Materials and Methods

A large dose of the standard laboratory strain of myxoma virus (Fenner and Marshall 1957) was inoculated intradermally in the shaved skin of the back of adult rabbits and the lesions allowed to develop for 6–7 days. *Aedes aegypti* mosquitoes which had been starved for several hours were then allowed to probe repeatedly for approximately 5 min on the primary lesions.

* Electron Microscopy Unit, John Curtin School of Medical Research, Australian National University, Canberra.

The head and mouthparts were then carefully removed and embedded in 1% agar to reduce the possibility of washing virus particles off during subsequent treatments. They were then fixed in either 1% buffered osmium tetroxide (pH 7·2) or 0·6% buffered potassium permanganate (Luft 1956) for 2 hr at room temperature, washed, dehydrated, and embedded in Araldite. Sections were cut with an L.K.B. Ultrotome microtome, mounted on carbon-coated grids, post-stained with uranyl acetate, and examined in a Siemens Elmiskop I electron microscope. Sections approximately 2μ thick were examined by phase-contrast microscopy.

A sample of infected rabbit skin, fixed in osmium tetroxide and embedded in Araldite, was kindly supplied by Dr. E. H. Mercer.

III. Results and Discussion

The feeding mechanism of the mosquito has been thoroughly worked out by many authors [see Gordon and Lumsden (1939) for example]. The six piercing stylets are held together by surface tension and act as one organ, the fascicle. It is the rapid sawing motion of the maxillae which makes a channel for the fascicle to follow, so it is not surprising that in this study the majority of virus particles were observed on the maxillae; the remainder were found on either the inner or outer surfaces of the labrum–epipharynx. However, the possibility that virus particles occur at other locations is not eliminated, as the preparative procedure may have dislodged many particles.

Typical examples of the appearance of the virus particles in sections of the maxillae are shown in Plate 1, Figures 2 and 3; these images may be compared with those of virus particles in an infected epidermal cell (Plate 2, Fig. 1). The permanganate treatment of the mouthparts destroys some of the detail seen in sections of particles fixed in osmium tetroxide but their characteristic membranous appearance is still preserved.

On one set of mouthparts examined, the number of particles in a series of adjacent cross-sections, taken about 0·5 mm from the tip, were observed. On average one virus particle was contained in any one section. Assuming that (1) the sections, which were a silver colour when viewed normally in reflected light, were approximately 500 Å in thickness (Peachey 1958), (2) about one-third of the length of the proboscis (0·7 mm for *A. aegypti*) is inserted on biting, i.e. this portion only contains the particles, and (3) the distribution is uniform, then the total number of particles on the stylets would be 14,000, the same order of magnitude as counts obtained by Fenner (see Fenner and Ratcliffe 1965) by grinding head and mouthparts and titrating the virus in rabbits and eggs. Probably the distribution of virus is not uniform, especially at the tip where the surface area is greater and where unfortunately particles were not seen, possibly because they were removed during preparation, so this value of 14,000 serves only to give an idea of the total count (infective plus non-infective particles).

On closer examination of Plate 1, Figure 3, and many similar micrographs, there appears to be an outer coat of the virus particle which has become detached from the particle but still lies close to the maxilla surface and separated from it by

a distance of approximately 100 Å. Whatever lies between these two surfaces is either not preserved or not stained by the permanganate–uranyl acetate procedure.

In all sections examined, very little cellular debris, except the virus particles themselves, was observed adhering to the surface of the stylets. One might suspect, then, that whatever is responsible for sticking virus particle to stylet originates from the particle itself. In a further attempt at resolving this problem, a centrifuged pellet of rabbit pox virus particles, which are morphologically identical to myxoma virus and all other members of the pox virus group, was fixed with osmium tetroxide, embedded, and sectioned as already described. Plate 2, Figure 2, shows a micrograph of such a section, post-stained with a lead tartrate complex solution (Millonig 1961). An irregular surface layer can be detected varying from 75 to 100 Å in extent and in some cases, such as that illustrated, the layers of adjacent particles come into contact and appear to merge into one another. Whether or not this layer is responsible for the adhesion of virus particles to stylet cannot be decided from such a purely morphological examination, but further studies on virus particles with chemically or physically modified surface properties could clarify this finding.

Whatever the nature of the bond (electrostatic, etc.), it is evident that it is not a particularly strong one; for the mosquito to be an efficient vector this bond must be easily made and broken.

IV. Acknowledgments

I wish to thank Dr. E. H. Mercer and Professor F. J. Fenner for helpful criticism and discussions, and Dr. K. B. Easterbrook for providing me with the rabbit pox virus suspension. This study was undertaken during the tenure of a CSIRO Senior Studentship.

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Explanation of Plates 1 and 2

Plate 1

Fig. 1.—Phase-contrast micrograph of a cross-section of the mouthparts of *Aedes aegypti*, about 2 mm from the tip. The mouthparts have become distorted during fixation and embedding and in life the fascicle lies within the labial gutter: *fc*, food canal; *hyp*, hypopharynx; *l–ep*, labrum–epipharynx; *lab*, labium; *lg*, labial gutter; *man*, mandibles; *max*, maxillae; *sal*, salivary duct; *sc*, cuticular scales. × 850.

Figs. 2 and 3.—Electron micrographs of myxoma particles on the surface of the maxillae. Both figures are of an area similar to the area outlined in Figure 1, but closer (c. 0·5 mm) to the tip. In Figure 3, the virus particle has been displaced from the maxilla surface, and what appears to be a surface coat on the particle has been detached (arrow). × 50,000.

PLATE 2

Fig. 1.—Section of portion of a myxoma cell showing the appearance of the virus particles in the dermis of the rabbit. A group of immature particles are shown at lower right. ×50,000.

Fig. 2.—Section through a pellet of rabbit pox virus particles. At higher magnification the outermost layer is irregular in appearance. The single arrow shows two particles where these layers have become partially fused. The double arrow indicates the extent of the layer (75–100 Å). ×270,000.

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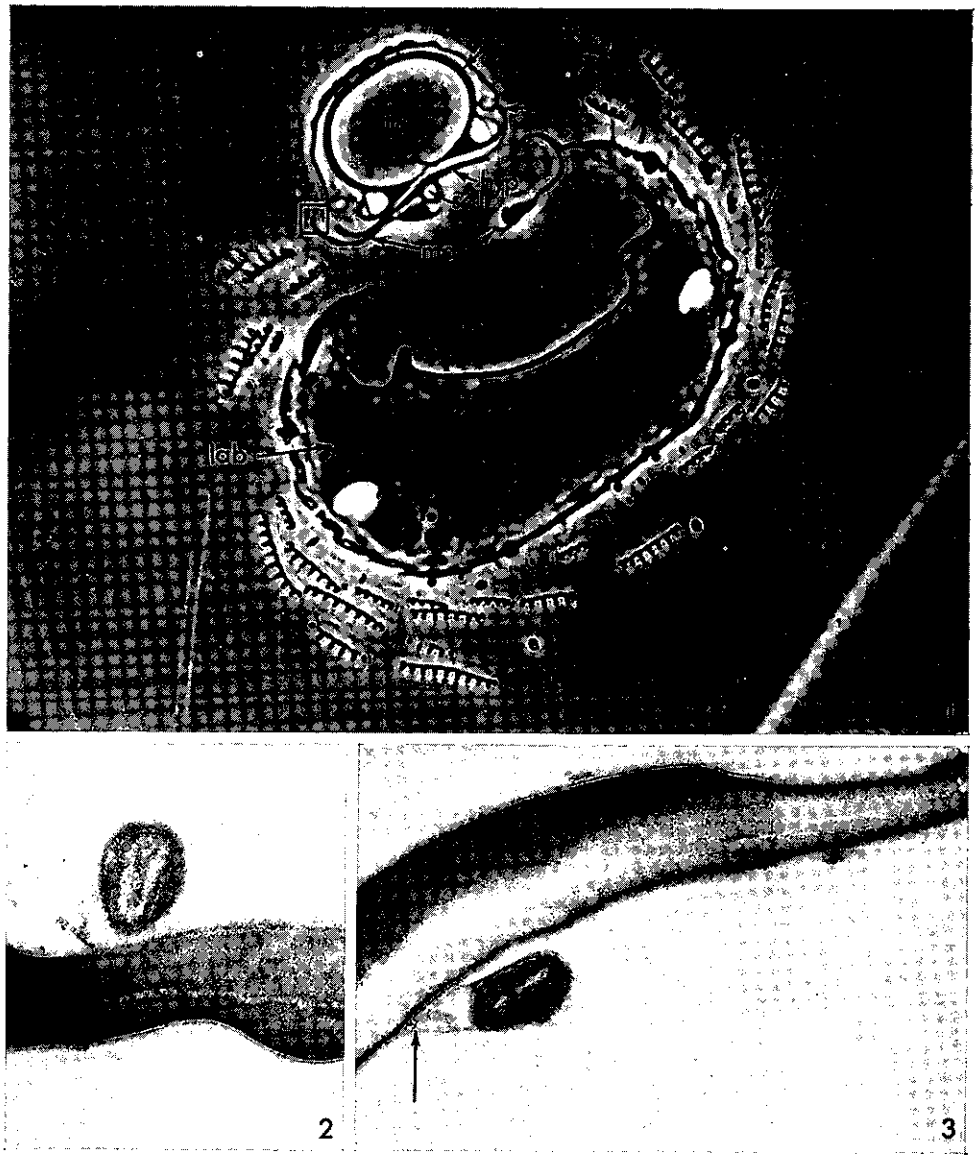

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