A Possible Infection Pathway in the Development of a Nuclear Polyhedrosis Virus

By K. A. HARRAP* AND J. S. ROBERTSON

Insect Pathology Unit, Commonwealth Forestry Institute, University of Oxford

(Accepted 19 March 1968)

SUMMARY

Larvae of the small tortoiseshell butterfly, Aglais urticae (Lepidoptera: Nymphalidae), were infected orally with a nuclear polyhedrosis virus. Sections of larvae which had been killed and fixed at intervals after infection were examined by light and electron microscopy. Typical virus development was observed in the fat body cells. However, in the nuclei of infected columnar cells of the mid-gut no incorporation of the virus particles into the polyhedral inclusion body was observed although both virus particles and crystalline polyhedron protein were present. Subsequently virus particles were found in the cytoplasm of these cells between the nucleus and the basal membrane. It is proposed that this unusual virus development is significant in the pathway of infection of a nuclear polyhedrosis virus.

INTRODUCTION

The development of nuclear polyhedrosis viruses in lepidopterous larvae is usually confined to the nuclei of haemocytes, cells of the fat body, hypodermis and the epithelial lining of the tracheae. The maturation of the virus within the nuclei of such cells has been described. (Smith & Xeros, 1953; Xeros, 1953, 1955, 1956; Bird, 1964). The form in which the virus infects these cells is not known, neither is the mechanism whereby this infection occurs. Nuclear polyhedrosis viruses do not normally develop in gut cells even though infection is initiated by oral ingestion of polyhedra.

METHODS

Light microscopy. Pieces of fat-body tissue and whole mid-guts were removed by dissection under Trager's B medium (Trager, 1935) and placed in Dubosq-Bouin fixative. The tissues were then dehydrated in a graded ethanol series, cleared in methyl benzoate, and embedded in Paraplast (Shandon Scientific Co. Ltd, 65 Pound Lane, Willesden, London, N.W. 1o.). Sections were stained with dilute Giemsa's stain.

Thick sections of tissue embedded in Epikote resin were cut with an L.K.B. ultramicrotome. These sections were stained in a 1:1 (v/v) mixture of hot 1% toluidine blue + 1% sodium borate.

Electron microscopy. Small pieces of gut and fat-body tissue were removed by dissection under Trager's B medium. Initial fixation was in 1% glutaraldehyde for 1 hr followed by 2 washes of 15 min. each and a subsequent fixation in 2% (w/v)

* In receipt of a Research Fellowship from Rentokil Ltd.
osmium tetroxide. Trager’s B medium was used both as a diluent for the fixatives and for the intermediate washes. After a further wash the tissues were dehydrated through a graded ethanol series and embedded in Epikote. Thin sections were cut on an L.K.B. ultramicrotome and stained in 2% (w/v) uranyl acetate followed by lead citrate (Reynolds, 1963). The sections were examined on an AEI EM 6B electron microscope at 60 kv using instrumental magnifications of \( \times 10,000 \) to \( \times 80,000 \).

**RESULTS**

**Light microscopy**

The first evidence of infection in fat-body tissue was detected 72 hr after ingestion of polyhedra. The nucleus lost its stellate shape and became rounded; it then enlarged considerably and as it did its granular chromatin disappeared and was replaced by virogenic stroma which filled it completely. As the infection progressed, the virogenic stroma appeared to draw away from the nuclear membrane, giving rise to the ‘ring zone’ stage (Smith & Xeros, 1953; Xeros, 1953, 1955, 1956). The development of small polyhedra could be discerned in the ring zone and within the virogenic stroma (Pl. 1a). The virogenic stroma decreased in size as more polyhedra were formed and eventually disappeared.

No similar evidence of virus infection or other abnormalities were seen in the nuclei of mid-gut epithelial cells (Pl. 4a). However, when corresponding thin sections were examined with the electron microscope, clear evidence of virus development was found. This could not be seen with the optical microscope as polyhedron protein and nuclear chromatin stained to the same intensity with toluidine blue.

**Electron microscopy**

Examinations of sections of fat-body tissue with the electron microscope showed that the development of the *Aglais urticae* virus was similar to that of other nuclear polyhedrosis viruses (Smith & Xeros, 1953; Bird, 1964). Visible infection of the fat body cells occurred 72 hr after ingestion of the virus. Initially, the nuclear content of the fat-body cells appeared as a mesh or network of strands from which naked virus particles (or nucleocapsids) were produced. The individual virus particles acquired a membrane in the spaces or lacunae within this mesh (Pl. 1b). A clear space was visible between the membrane and the enclosed virus particle. The enclosed virus particle was often seen to be curved or sometimes bent almost in two. Cross-sections of such bent forms erroneously suggested that 2 virus particles could be enclosed by the same membrane. There are many examples of nuclear polyhedrosis viruses where several virus particles are bounded by one membrane. However, with the *A. urticae* virus,

---

**PLATE I**

Tissue sections of *Aglais urticae* larvae.

(a) Photomicrograph of infected fat body. The condensed nuclear content in the infected cells is actually virogenic stroma (v.s). Immature polyhedra (i.p.) are developing in some cells.

(b) Electron micrograph of the nucleus of an infected fat-body cell. The strands of the virogenic stroma (s.v.s.) give rise to virus particles which acquire membranes (v.p.m.) in the spaces within and around the network.
cross-sections of mature polyhedra and studies on virus particles extracted from polyhedra by alkaline treatment (Harrap & Juniper, 1966) have shown that the particles occur singly.

The next stage was the crystallization of the polyhedron protein which incorporated the membrane-bounded virus particles. This process can apparently start when as few as three particles are in close proximity. Identification of the early crystallization of the polyhedron protein was possible as the regular cubic lattice structure was easily seen in section. As the deposition of the polyhedron protein proceeded further, adjacent virus particles were surrounded and the polyhedron increased in size. During this process, the membranes loosely surrounding the virus particles were compressed. However, not all the virus particles produced in the nucleus were incorporated into polyhedra (Pl. 2, 3). In cross-sections of the mature polyhedra it was sometimes possible to detect an outer membrane.

Virus development was first seen in the nuclei of columnar cells of the mid-gut epithelium 24 hr after infection though the process was different from that observed in the fat body cells. Development of virus was not seen in the goblet cells of the mid-gut. The network of chromatin strands seen in infected fat-body cells was not found but aggregations of dense nuclear material were present. Virus particles with and without membranes were distributed throughout the nucleus though in far fewer numbers than in the infected fat-body cells (Pl. 4b). Discrete bodies resembling polyhedra were seen and closer examination showed these to be crystallized masses of polyhedron protein as the regular lattice structure was clearly visible (Pl. 4c). No incorporation of the virus particles into the polyhedron protein had occurred although a few were found on the periphery of these masses. The formation of polyhedra containing virus particles evidently did not occur. Examination of the cytoplasm of these infected columnar cells showed virus particles lying between the nucleus and the basal cell membrane (Pl. 4d). The epithelial cells of the mid-gut are situated on a basal lamina (Anderson & Harvey, 1966) and groups of virus particles have been seen in folds of the lamina. Individual particles have also been found within the basal lamina. Muscle tissue and tracheoles with the adjoining tracheolar cells are situated beneath the basal lamina. The tracheolar cells were frequently found to be infected with virus from 48 hr after infection feeding. Polyhedra containing virus particles were formed in such infected cells.

DISCUSSION

The polyhedron can be logically regarded as an effective means of protection for the infective virus particles because it is extremely resistant to physical and chemical treatment, it remains infective after years of storage (Bergold, 1958; Steinhaus, 1960) and the virus particles incorporated into it can have no further infective function in the same host larva. Individual virus particles therefore must initiate nuclear polyhedrosis virus infection and be responsible for its subsequent progression.

PLATE 2

Tissue section of Aglais urticae larvae.

Electron micrograph of the nucleus of an infected fat-body cell. Mature polyhedra (m.p.) contain many virus particles. Some virus particles (v.p.) are still present in association with the remaining network.
In fat-body cells, virus particles not incorporated into polyhedra are available to infect adjacent cells. The significance of nuclear polyhedrosis virus development in the columnar cells of the mid-gut epithelium, however, is that all the virus particles in these nuclei are potentially capable of infecting other cells of the same larva as no incorporation of the virus particles by the crystalline polyhedron protein takes place. The reasons for this lack of incorporation during virus development in the columnar cells are not yet clear. It has been shown by fluorescent-antibody studies that polyhedron protein is produced in the cytoplasm during the early stages of infection of the cell (Krywienczyk, 1963) and it is possible that in the nucleus the membranes of the virus particles act as a substrate for its crystallization. The number of adjacent virus particles around which this crystallization can proceed may determine the size of the polyhedron. In a region of polyhedron protein crystallization in columnar cells the number of virus particles with membranes may be too few for incorporation to progress. This is very possibly because virus particles are continually migrating into the cytoplasm. A further possibility is that the membranes of the virus particles are modified in some way during their development in columnar cells so that incorporation into polyhedra cannot occur.

There appears to be a border of short projections on the outside of the membranes of the virus particles in the basal cytoplasm of a columnar cell (Pl. 4d). This border could represent a modification of the virus membrane as it is not seen in those particles that have developed in, for example, fat-body cells. However, this border is not found consistently on virus particles in gut cells, and furthermore other membranous structures in the cytoplasm, of doubtful virus origin, also possess it.

The virus particles released from the nucleus of a columnar cell are possibly moving along a gradient in the cell cytoplasm between the nucleus and the basal cell membrane. The distribution of the cell organelles does not conflict with this suggestion. Support for this idea is also provided by the probable absorptive function of the columnar cells in contrast to the secretory function of the goblet cells (Anderson & Harvey, 1966) where no virus development was observed. Virus particles were seen not only in the basal cytoplasm of the columnar cells but also grouped in folds within the basal lamina. It seems clear that virus particles produced in the columnar cells of the gut are responsible for further infection of the host as cells of the tracheal epithelium adjacent to the basal lamina were found to show various stages of virus development. The mechanism of initial infection of columnar and other cells of susceptible tissues is not yet clear. Attempts are being made to determine this process and to confirm the proposed pathway of infection in other nuclear polyhedrosis virus diseases.

The electron microscope and its ancillary equipment was purchased with a grant from the Natural Environment Research Council.

PLATE 3

Tissue section of *Aglais urticae* larvae.

Electron micrograph of developing polyhedra in a fat-body cell. Crystallizing polyhedron protein (p.) surrounds the virus particles. Compression of the virus particle membrane is apparent during this process.
REFERENCES

(Received 6 November 1967)

---

PLATE 4
Tissue sections of *Aglais urticae* larvae.
(a) Photomicrograph of columnar cells (c.e.) and goblet cells (g.c.) of the mid-gut epithelium. Infection of the columnar cell nuclei is not apparent.
(b) Electron micrograph of infected columnar cell nuclei. Both virus particles (v.p.) and masses of crystallized polyhedron protein (p.p.) are present. Villi of an adjacent goblet cell (v.g.c.) can also be seen.
(c) Enlargement of the masses of crystallized polyhedron protein in the nucleus of the infected columnar cell in (b). The regular lattice structure is clearly visible.
(d) Virus particles (v.p.) in the cytoplasm of an infected columnar cell.