THE STRUCTURE OF A POLYHEDRAL VIRUS FROM THE LARVA OF *ARDICES GLATIGNYI* LE GUIL. (LEPIDOPTERA: ARCTIIDAE)

by

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The structure of the inclusion bodies associated with nuclear polyhedrosis in insects has been described by a number of workers. The earlier studies, which have been summarised by BERGOLD[1], depended on alkali treatment to liberate the virus particles from mature polyhedral bodies. Recent workers[4,7,11,13], have used the electron microscope to study thin sections of infected tissue, a method which provides more reliable and direct evidence on both structure and development of the polyhedra.

In this paper the structure of polyhedral bodies found in larvae of *Ardices glatignyi*, is described. Both thin sections and polyhedra disrupted by alkali treatment were examined. The results, in general, confirm the findings of others, particularly those of HUGHES[7], but do not wholly support published views[12] on the mechanism of virus reproduction.

MATERIALS AND METHODS

Larvae of *A. glatignyi* are found on the plains around Adelaide, South Australia, from May or June until mid-October. Signs of the virus disease appear most commonly in the last two larval instars, during August, September and October. Larvae with an advanced infection are easily detected by changes in their behaviour. They do not immediately release their hold on the host plant when disturbed but cling firmly, and if dislodged, are unable to curl up in the usual protective posture. Finally the larvae become sluggish, climb to the top of a branch or leaf on the host plant and attach themselves firmly. Death ensues in 2–3 days. Within another 4–5 days the body wall ruptures, releasing a brownish white fluid laden with polyhedral bodies. Optical examination of paraffin sections kindly prepared by Dr. M. F. DAY, showed that the polyhedra were present in the nuclei of cells of the epidermis, fat body, tracheae and blood.

A suspension of polyhedra was prepared as follows. Diseased larvae were collected in the field and maintained at 25° C until two days after death. The contents of the body cavity were then drawn off, diluted with distilled water, and centrifuged at about 500 r.p.m. for five minutes to remove tissue debris. The supernatant liquid was spun at 3,000 to 5,000 r.p.m. to throw down the polyhedral bodies which were then washed several times with distilled water.

Specimens of isolated polyhedra were prepared for electron microscopy by drying drops of a suitably diluted suspension on collodion film mounted on object carriers in the usual way. Some of these were examined without further treatment in order to check the purity of the suspension. The size of the polyhedra was also measured on these untreated preparations.

Others, after drying, were exposed to the action of large drops of 4% Na_2CO_3 solution, (SMITH AND WYCKOFF[12], DAY *et al.*[5]), for 5 to 10 minutes in a saturated atmosphere at room temperature (20–24° C). Under these conditions evaporation from the drops of Na_2CO_3 solution was negligible. The alkaline solution was removed with filter paper and the specimens washed

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by repeated application of drops of distilled water and their subsequent removal with filter paper. When dry, after the final wash, the specimens were shadowed with palladium to give a shadow-ratio of about 3:1.

For the preparation of thin sections, tracheae were taken from larvae in the earliest stage of the disease which could be recognised in the field. However, at this stage nuclear infection was already extensive. Living tracheal tissue was fixed, and embedded in methacrylate according to the method of PALADE[10]. Sections were cut with a microtome similar to that described by HODGE *et al.*[6], and these specimens examined without removing the methacrylate. Micrographs of all preparations were taken with a Philips Electron Microscope operated at 60 kV and calibrated for magnification with polystyrene latex particles.

RESULTS

The isolated polyhedra were similar in appearance to those from other species of insects. They ranged in size from about 1.0 to 2.5 μ across the largest dimension.

Treatment with 4% Na_2CO_3 solution for 5 minutes was sufficient to disrupt the polyhedra and release bundles containing virus rods and also individual virus particles (Figs. 1 and 2). The particles were about 310 mμ long and 50 mμ wide, but the latter figure is an over-estimate because the rods undoubtedly flattened as they dried on the collodion film. In micrographs of polyhedra which had been treated with alkali it was difficult to assess accurately the number of rods in a bundle because rods may have been lost, or added from other bundles during treatment. The number seemed to vary from 5 to 8 or more. BERGOLD found distinct developmental membranes surrounding the bundles of rods in other polyhedra but our micrographs did not reveal similar structures. If such membranes had existed they may have been destroyed by the rather strong alkaline solution used for treatment. On the other hand the developmental membranes studied by BERGOLD AND WELLINGTON[3] were resistant to concentrations of alkali sufficient to destroy the virus substance. The intimate membranes which surround individual rods, as shown in Fig. 1, resemble

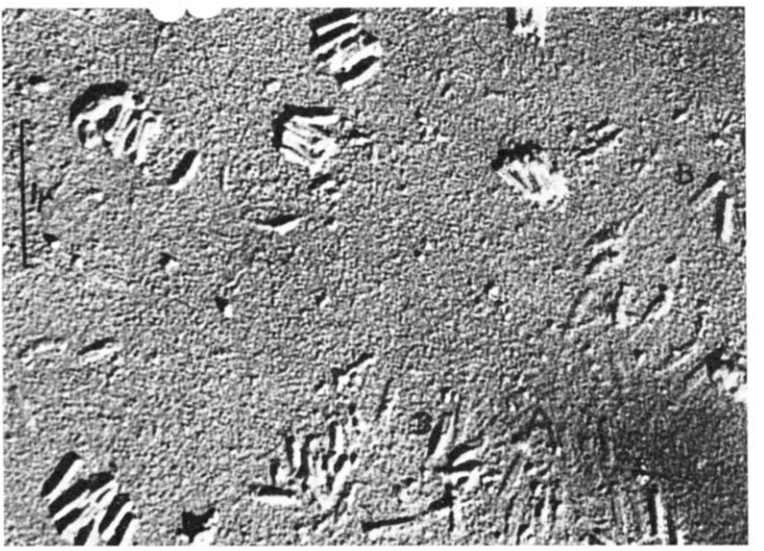

Fig. 1. Virus particles released from polyhedra of *Ardices glatignyi* by treatment with sodium carbonate solution. Bundles of virus rods in various stages of disintegration are to be seen. Intimate membranes occur at A and partially destroyed rod at B. Magnification 19,000 ×.

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Fig. 2. Preparation as in Fig. 1. Bundles broken down to individual rods, some of which appear to have tails as indicated by arrows. Magnification 19,000 ×.

those found by BERGOLD[1,3]. There is some evidence of breakdown of the rods, within the intimate membranes, into more or less spherical sub-units, an effect also observed by BERGOLD. Structures resembling "tails" appeared on some rods (Fig. 2), but this and the appearance of spherical sub-units may be artifacts resulting from the treatment with alkali.

In micrographs of sections the nuclei of infected tracheal cells were seen to contain many polyhedral bodies apparently in various stages of formation (Figs. 3, 4). In Fig. 3 the formation of polyhedra is far from complete whereas in Fig. 4 they are more nearly mature. The general appearance of the nuclei agrees with descriptions from optical microscopy in that the developing polyhedra are arranged around the central mass of chromatin (Fig. 3). Within this peripheral region many rod-shaped virus particles can be seen, at this stage of the infection, aggregated into bundles of two or more rods. Some micrographs showed cross-sections of virus bundles, as at A in Fig. 3, and from several of these it appeared that the number of rods in a particular bundle varied between 4 and 8 at least and that they occurred in an ordered parallel array. Usually, these bundles were not obviously surrounded by a membrane. However, a structure resembling an envelope was occasionally seen. Figs. 3 and 4 show that the virus bundles aggregated with some non-virus material to form polyhedra, throughout which they are irregularly distributed. The marked osmiophilia of sectioned polyhedra might suggest the presence of considerable amounts of lipoid. However, BERGOLD[2] found little or no fat in similar polyhedra from other species.

In addition to identifiable virus particles, fine fibrous material was distributed through the nucleus. It occurred within and around the chromatin network, but the latter did not appear to have a fibrous structure. It is not known if this material was involved in virus formation but attention is drawn to it in view of the work of SMITH AND XEROS[13] who suggested that a fibrous component of the chromatin was converted into virus particles.

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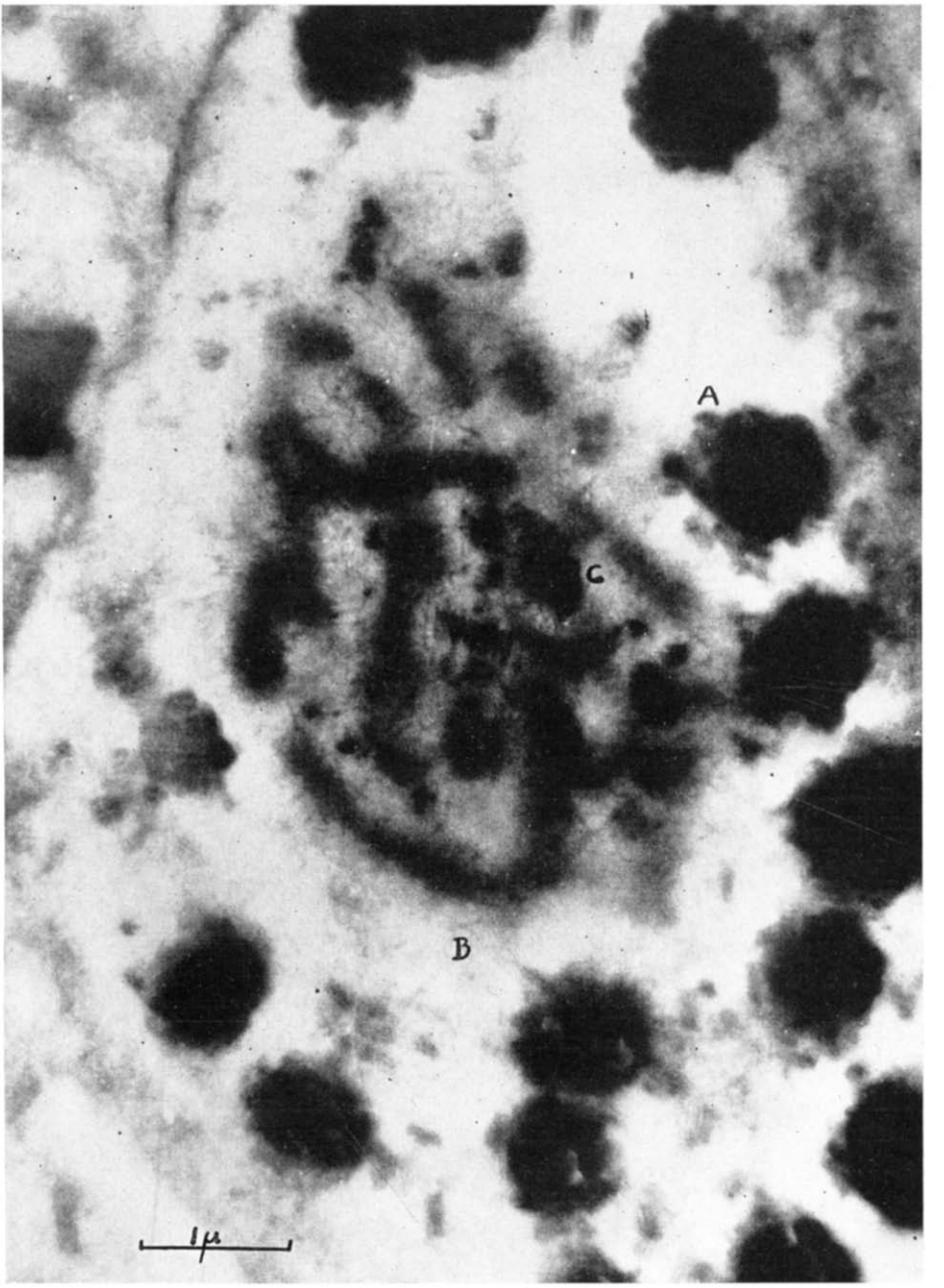

Fig. 3. Section of infected nucleus of tracheal cell. The central chromatin net, bundles of virus rods and developing polyhedra are conspicuous. At A is a cross-section of a bundle of four rods. Around B fine fibrous material can be seen. This appears throughout the central region of the nucleus. C is an unidentified structure which may be a nucleolus. Magnification 19,000 ×.

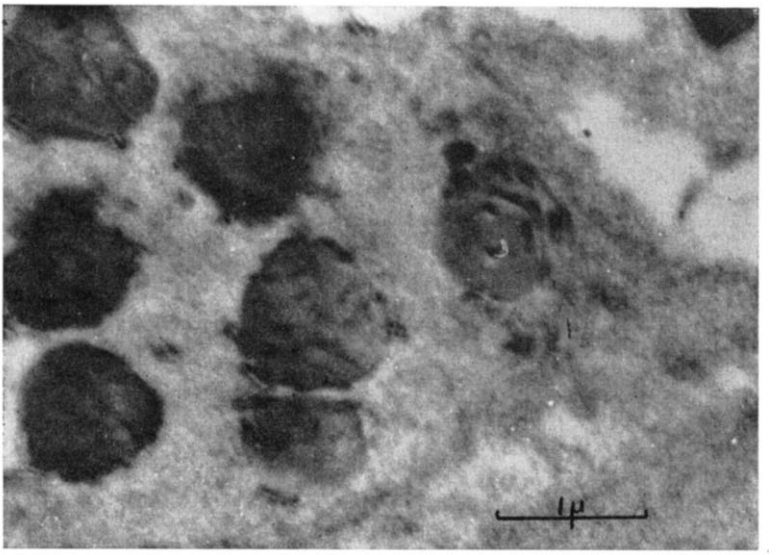

Fig. 4. Section of infected nucleus of tracheal cell. The polyhedra are more mature, and here the irregular arrangement of bundles of rods within the polyhedra is more apparent. Magnification 10,000 ×.

DISCUSSION

The observations reported here on the morphology of isolated polyhedra from *A. glatignyi* are in substantial agreement with the findings of other authors using other material and the work on sections of infected cells confirms the results which HUGHES[7] obtained in a more extended study of *Borrelina campeoles* Steinhaus.

The most important point for discussion is the process of virus multiplication. From the micrographs of sections shown in this paper and from those of HUGHES[7] it seems clear that the virus particles were fully formed before they entered the developing polyhedra. In later stages the rods formed bundles which were sometimes seen to be surrounded by membranes. Finally, these bundles of rods, were incorporated into polyhedra together with material which in other viruses BERGOLD found to be protein. Apparently the multiplication of the virus particles occurred solely in the nuclear sap. This conclusion is opposed to the hypothesis of BERGOLD[1,2] who postulated that rods were formed within developmental membranes from spherical precursors, and that all stages of such a process were present in mature polyhedra. He further postulated that the rods once formed may also break down into spherical sub-units which then give rise to new rods.

The evidence so far provided by micrographs of sections gives no indication of the mode of origin of single rods. No spherical forms were found either in our sections or those of HUGHES[7], although such bodies, apparently derived from rods during alkali treatment, have been reported by BERGOLD and others. This however is not sufficient evidence that the spheres are the precursors of the rods. Indeed BERGOLD[1] found that suspensions of these spheres were less infectious than suspensions of intact rods. Our observations and those of HUGHES[7] on polyhedral viruses in Lepidoptera suggest that BERGOLD's hypothesis should be treated with reserve.

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An alternative mode of virus multiplication has been proposed by SMITH AND XEROS[13] who suggested that the rod-shaped virus particles may be derived from a fibrous component of the central chromatin net which appears in infected nuclei. The two micrographs in their paper do not clearly demonstrate such a component but do show individual virus rods projecting from a dense chromatin mass and lying near it. HUGHES[7] considered that his micrographs gave no indication of fibrous material in the chromatin. Our observations, though less extensive, do show as in Fig. 3, fibrous material in the nucleus in the region of the chromatin net. But whether this is to be regarded as a component of the chromatin, or whether it has any connection at all with the multiplication of the virus, cannot be decided without further investigation.

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SUMMARY

The virus causing a polyhedrosis in the larva of the arctiid moth *Ardices glatignyi* Le Guil. has been studied by electron microscopy of both isolated polyhedra and sections of infected tissue. In general, the findings agree with those of other workers notably BERGOLD[1] and HUGHES[7] but the observations on infected tissues do not confirm BERGOLD's hypothesis of the multiplication of the virus. The virus rods appear to develop completely in the nuclear sap and subsequently to aggregate in bundles, which then become embedded in other material to form the polyhedra. There is no regular pattern in the distribution of the bundles within the polyhedra. How the rods originate remains to be determined.

RÉSUMÉ

Le virus qui provoque une polyédrose chez la chenille de la phalène *Ardices Glatignyi* Le Guil. a été étudié par microscopie électronique à la fois des polyèdres isolés et de sections de tissu infecté. En général, les résultats concordent avec ceux d'autres auteurs notamment BERGOLD[1] et HUGHES[7] mais les observations sur tissus infectés ne confirment pas l'hypothèse de BERGOLD sur la multiplication du virus. Les bâtonnets de virus semblent se développer entièrement dans le suc nucléaire et se réunissent ultérieurement en amas, qui sont ensuite enveloppés dans une substance différente pour donner les polyèdres. Il reste à déterminer comment prennent naissance les bâtonnets.

ZUSAMMENFASSUNG

Der Virus, der bei der Raupe des Falters *Ardices glatignyi* Le Guil. die Vielflächnerkrankeit verursacht, wurde in isolierten Vielflächnern und Sektionen von infiziertem Gewebe mit Hilfe des Elektronenmikroskopes untersucht. Im Allgemeinen stimmen die Ergebnisse mit denjenigen anderer Forscher, wie BERGOLD[1] und HUGHES[7], überein; die mit infizierten Geweben gemachten Beobachtungen bringen jedoch keine Bekräftigung von BERGOLDS Hypothese über die Virusfortpflanzung. Die Virusstäbchen scheinen sich vollständig im Zellkernsaft zu entwickeln, um sich nachher zu Bündeln zusammenzuballen, welche dann von anderem Material umgeben werden und so Vielflächner bilden. Es besteht kein regelmässiges Muster bei der Verteilung der Bündel im Inneren der Vielflächner. Der Ursprung der Stäbchen muss noch aufgeklärt werden.

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REFERENCES

1 G. H. BERGOLD, *Advances in Virus Research*, K. M. SMITH AND M. A. LAUFFER (editors), Academic Press Inc., New York, Vol. 1, p. 91, 1953.
2 G. H. BERGOLD, *The Nature of Virus Multiplication*, P. FILDES AND W. E. VAN HEYNINGEN (editors), Camb. Univ. Press, Cambridge, p. 276, 1953.
3 G. H. BERGOLD AND E. F. WELLINGTON, *J. Bacteriol.*, 67 (1954) 210.
4 F. T. BIRD, *Biochim. Biophys. Acta*, 8 (1952) 360.
5 M. F. DAY, I. F. B. COMMON, J. L. FARRANT AND C. POTTER, *Australian. J. Biol. Sci.*, 6 (1953) 574.
6 A. J. HODGE, H. E. HUXLEY AND D. SPIRO, *J. Hist. and Cytochem.*, 2 (1954) 54.
7 K. M. HUGHES, *Hilgardia*, 22 (1953) 391.
8 K. M. HUGHES, *J. Bacteriol.*, 59 (1950) 189.
9 P. LEPINE, C. VAGO AND O. CROISSANT, *Ann. Inst. Pasteur*, 85 (1953) 170.
10 G. E. PALADE, *J. Exptl. Med.*, 95 (1951) 285.
11 K. M. SMITH, *Science Progress*, 41 (1953) 411.
12 K. M. SMITH AND R. W. G. WYCKOFF, *Research*, 4 (1951) 148.
13 K. M. SMITH AND N. XEROS, *Nature*, 172 (1953) 670.

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