Insect viruses and their structure

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Virus research has tended to be concentrated upon forms that cause disease in man or in the crops and animals on which he depends. But such practical considerations are not necessarily important in fundamental research: for this purpose certain insect viruses, not hitherto very closely studied, seem to have important possibilities which are here discussed. A virus which infects larvae of *Tipula paludosa* has particularly interesting properties, and an ingenious shadow-casting technique has made it possible to identify its particles as icosahedra: it is the first virus in which the geometrical form of the particles has been demonstrated.

Viruses and virus diseases have been investigated by people whose aims have been fairly readily distinguishable as humanitarian, economic, or fundamental. The first aim involves consideration of the virus ailments of man and their alleviation, while the second is concerned with study of the diseases of the higher plants and animals and with reducing the losses that result from these diseases. The viruses that affect insects have not received much attention from those whose approach is either humanitarian or economic, and consequently they are not well understood from this point of view. But the third approach—the fundamental study of viruses—is concerned only with the questions of what viruses are and how they multiply within living cells. Here one kind of virus is as good as another if it promises to provide significant information. In this type of study, insect viruses may be important, and some of their known properties lead us to believe that recognition of their importance in fundamental virus research is overdue.

Although viruses attack almost every known kind of organism, there seem to be some exceptions. For example, in the plant kingdom no virus has been discovered that infects the ferns or the true fungi. In the insect world only three orders are definitely known to be susceptible to virus infection. This apparent restriction in host range may, in part, be only a reflection of our lack of knowledge, but it is so far the fact that viruses have been found only in the Lepidoptera (butterflies and moths), the Hymenoptera (ants, bees, and wasps), and the Diptera (the true flies). No virus diseases have been reported for the populous orders like the Hemiptera, which includes the aphids and plant bugs; the Orthoptera, such as grasshoppers and locusts; and the Coleoptera, containing some forty thousand species of beetles.

For practical reasons it might be distinctly important to discover virus diseases in some of the insect orders not now believed to harbour them. The deliberate inducing of virus plagues among insects has been studied for some time in the hope of exercising biological control over insect pests. Such attempts involve first collecting large quantities of some particular virus and then spraying the infectious material in trial fields where the appropriate insect pest is in the susceptible stage. Considerable success in such trials has been occasionally achieved [1], with dramatic mortality among the pests and no damage to the crops. But one of the drawbacks to the general use of this form of control is the existence of several kinds of insect pests, such as aphids and locusts, that appear to have no natural virus enemies.

KINDS OF INSECT VIRUSES

Since the symptoms displayed by insects suffering from virus diseases are rather variable and non-specific, the classification of insect viruses has been based upon their morphological and cytological characteristics. The characteristics used relate either to the appearance of the virus particles themselves, as seen under the electron microscope, or to the appearance and intracellular localization of certain inclusion bodies associated with the virus particles. On the latter basis three groups of insect viruses are now recognized, although one of the groups is commonly divided into two sub-types. The virus particles associated with any one group or sub-type seem to have a characteristic size and shape. In the few cases where the particles have been purified and analysed it has been found that they are nucleoproteins, with the nucleic acid of the deoxy type [2]. The types of disease produced by the viruses of a group seem to depend upon where the virus multiplies within the body of the host. Although there are instances of virus being recovered from

insects in the pupal, and even adult, stage [3], it is generally conceded that only the larval forms are susceptible to frank virus diseases. It is found that within the larvae many organs and types of cells are liable to infection by some virus of one of the three groups. The cells most commonly invaded are those of the skin, the tracheae, the fat-body, the mid-gut, and the circulating cells of the blood.

Polyhedroses. The largest of the three groups of insect viruses so far investigated is the one causing the polyhedral diseases, or polyhedroses. This name derives from the observation that large numbers of bodies of polyhedral shape are present within certain tissues of the infected host. In a few cases these bodies have been shown to be crystalline, as examined by X-ray analysis [4] and by electron microscopy [5]. The polyhedral crystals are not uniform in shape and size, but they are sufficiently characteristic in appearance, and large enough, to be easily recognized under the light microscope. For many years there was much controversy about the nature of the polyhedral bodies, and for some time they were generally thought to be some kind of micro-organism causing the disease. It is now known that the bodies are primarily protein crystals within which several hundred virus particles are occluded. We shall refer to true virus crystals later, and it should be noted that the polyhedral bodies, though crystalline, contain virus particles only as a minor constituent.

The polyhedral virus group is divisible into two types, in each of which the polyhedral bodies have characteristic properties and unique localization within infected cells. The nuclear polyhedral viruses usually attack the skin, tracheae, fat-body, or blood cells, and as their specific name implies, they are found to develop only in the nuclei of the cells affected. When the affected cells become grossly infected they burst and release the polyhedral bodies into the blood. These released polyhedral bodies are readily obtained in pure form by gentle sedimentation and decantation of a water suspension of the contents of the bodies of infected insects. When the polyhedra are treated with weak alkali the protein contained in them dissolves, leaving behind what appears to be an outer membrane within which are rod-shaped virus particles. They may be found either singly or in bundles (figure 1). It is possible to obtain the rod-like particles quite pure, and when this is done they are found to be highly infectious when inoculated into healthy larvae [6].

The cytoplasmic polyhedroses have been found only in the cells of the gut, usually the hind-gut, where they seem to develop solely in the cytoplasm of the affected cells. The polyhedral bodies may be extracted from the cells and purified; when this is done, the purified material proves to be infectious [7]. The morphological identification of the elementary infectious particle is not, however, as clear-cut as it is in the case of the nuclear polyhedroses. When the cytoplasmic polyhedral bodies are treated with alkali they partially dissolve, leaving behind a sort of matrix honeycombed with round holes, and a number of small, spherical particles [8]. Since these particles have not been assayed for infectivity it cannot be stated with assurance that they are the elementary infectious agent. The electron microscope reveals in thin sections of the cytoplasmic polyhedra the presence of small, approximately circular particles (figure 2) that are probably of the same nature as those extractable by weak alkali [9]. Inasmuch as sections cut through nuclear polyhedra show characteristic particles known to be viral in nature, it can be postulated, but only by analogy, that the small circular objects seen in sections of cytoplasmic polyhedra are the virus particles.

The two types of polyhedral virus disease differ in the symptoms they produce, particularly in lepidopterous larvae. The nuclear polyhedral viruses usually attack the skin, rendering it extremely thin and fragile; it ruptures at a touch and liberates the liquefied contents of the body, which consist largely of polyhedral crystals. Caterpillars that have died from a nuclear polyhedrosis are usually found in a characteristic position hanging by one or two pairs of the abdominal 'feet'. Infected caterpillars of the nun moth (*Lymantria monacha*) tend to migrate to the tops of spruce trees (their usual habitat), where they remain hanging head downwards. This rather peculiar behaviour has given rise to the German name of *Wipfelkrankheit*, or tree-top disease. In the disease produced by the cytoplasmic polyhedroses the skin is not attacked. The caterpillar dies intact and tends to dry up rather than liquefy. Towards the termination of the disease, in some cases, the cells of the gut are crammed with polyhedra and finally burst, spilling out the contents to be voided with the faeces. Frequently it is possible to see the polyhedra showing through the skin as white patches.

Granuloses. A second group of insect viruses is responsible for the diseases known as 'granuloses', a name deriving from the fact that the elementary virus particles appear to be enclosed in a

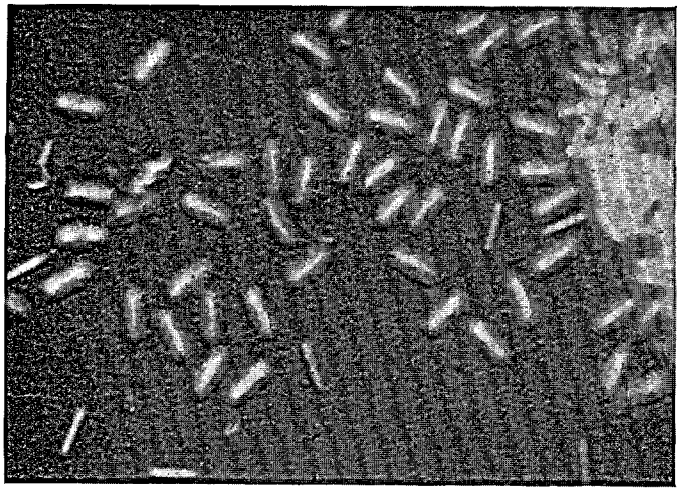

FIGURE 1 – *Nuclear polyhedral body from* Bombyx mori, *dissolved to disclose virus particles. Many are still enclosed within a membrane.* (× 20 000)

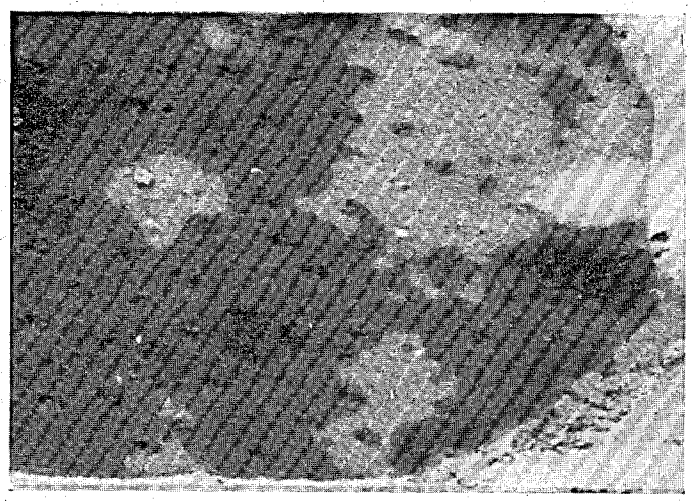

FIGURE 2 – *Section of cytoplasmic polyhedral body from* Arctia caja. *The quasi-circular bodies within are presumed to be sectioned virus particles.* (× 85 000)

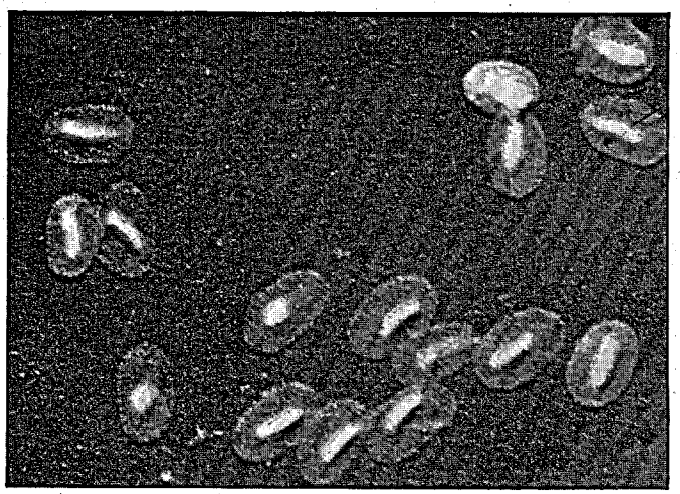

FIGURE 3 – *'Granules' from a granulosis disease of* Euplexia leucipera *after treatment with alkali. Note central capsules and flattened granules. The virus particle is inside the central capsule.* (× 26 000)

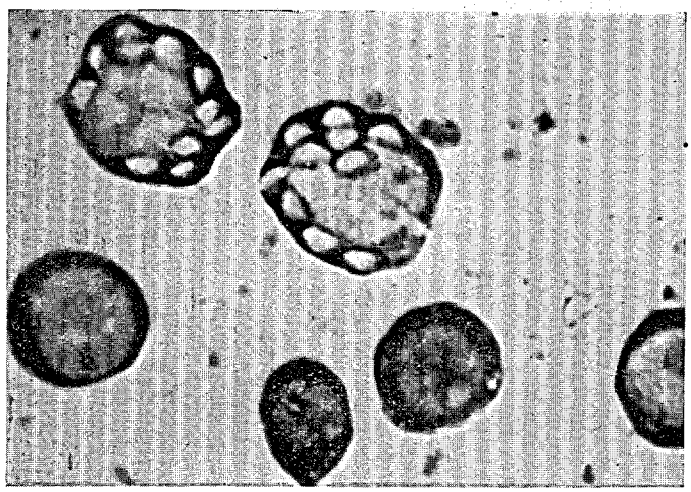

FIGURE 4 – *Blood cells of* T. paludosa *infected with a nuclear polyhedrosis. Upper two cells contain many polyhedral bodies. Light micrograph.* (× 1500)

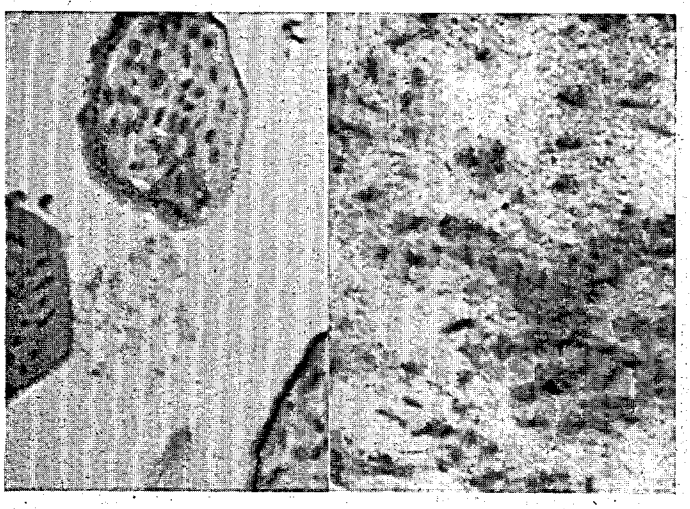

FIGURE 5 – (*left*) *Sections of polyhedral bodies from* T. paludosa, *containing rod-shaped particles* (× 24 000). (*right*) *Portion of nucleus showing similar particles, not enclosed in polyhedral body* (× 30 000).

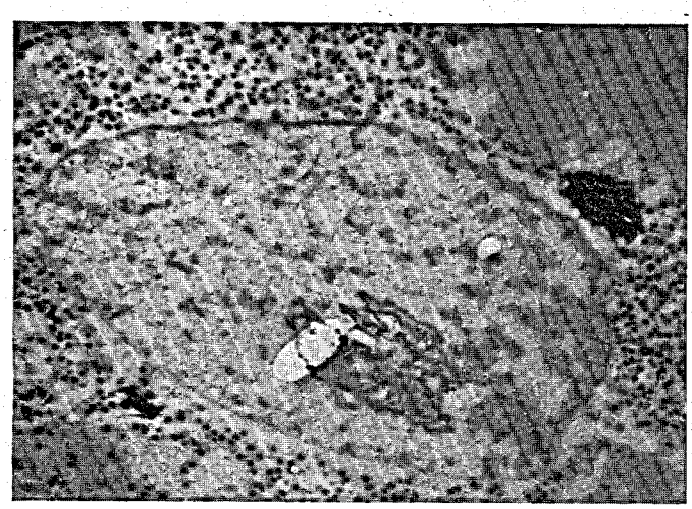

FIGURE 6 – *Portion of fat-body cell infected with* Tipula *iridescent virus (TIV). The darkly staining virus particles are solely in the cytoplasm.* (× 8500)

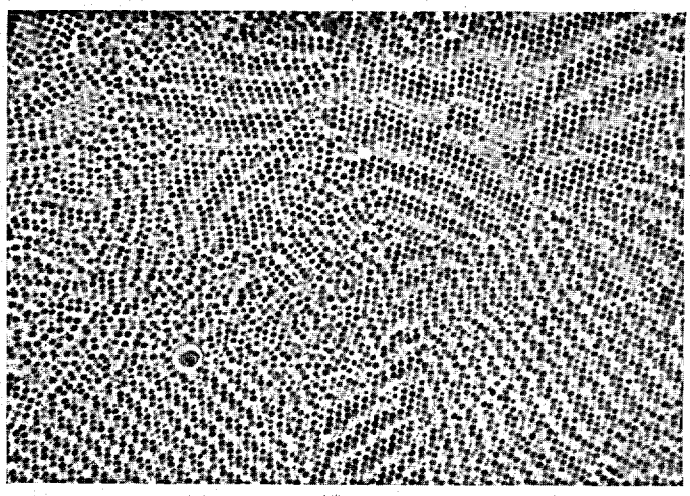

FIGURE 7 – *Pellet of TIV sectioned to show the existence of a polycrystalline array.* (× 10 000)

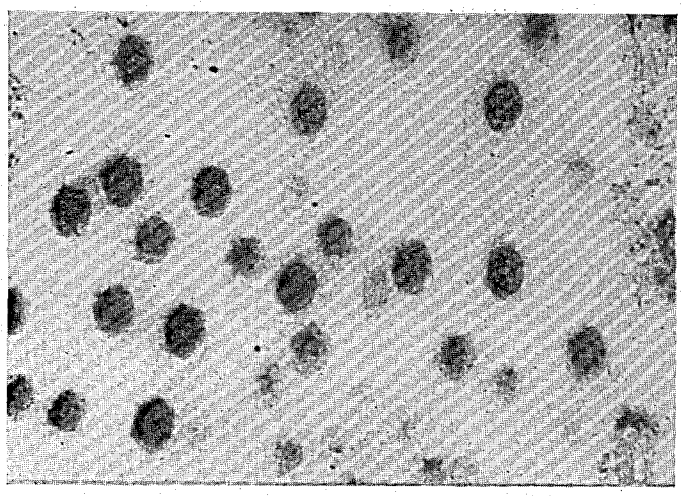

FIGURE 8 – *Sectioned particles of TIV, exhibiting some internal structure and frequent six-sided forms.* (× 55 000)

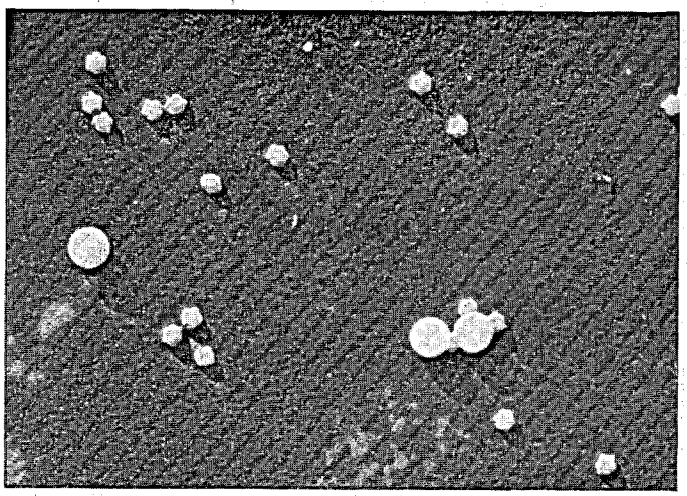

FIGURE 9 – *Air-dried shadowed particles of TIV. Note six-sided contours. Spherical objects are polystyrene latex particles for reference.* (× 23 000)

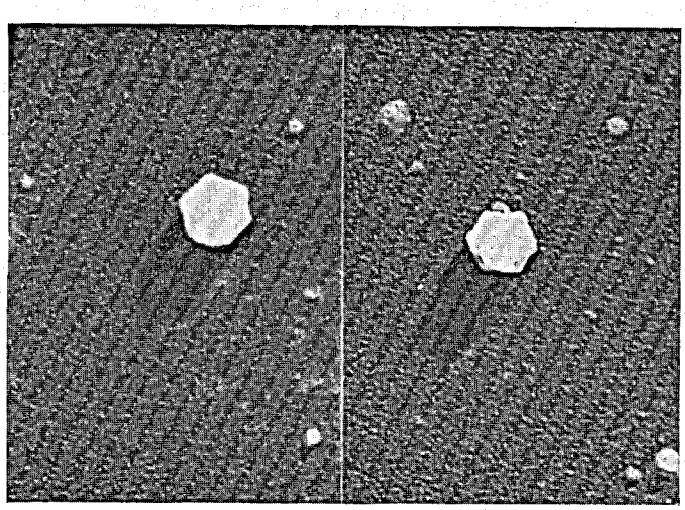

FIGURE 10 – *Two frozen-dried particles of TIV, showing that two distinct shadow forms may be cast: four-sided and pointed, five-sided and blunt.* (× 67 000)

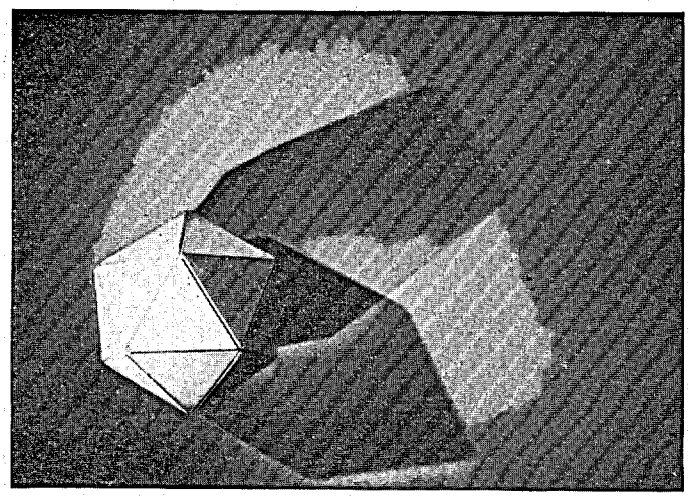

FIGURE 11 – *Model of icosahedron, doubly shadowed. Note two shadow shapes which can be cast simultaneously only by an icosahedron in this orientation.*

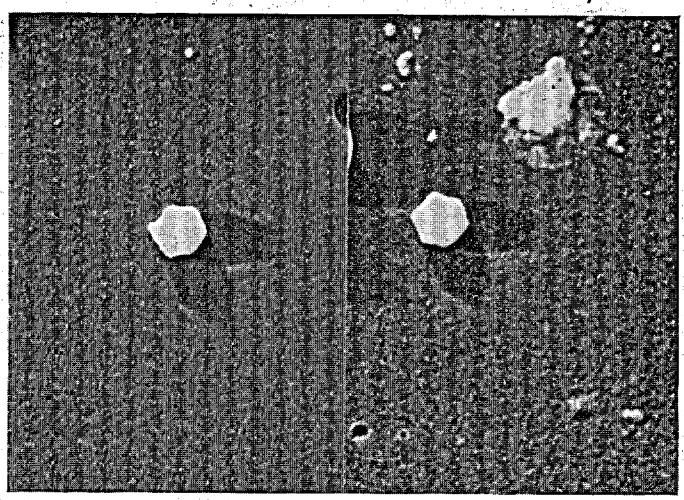

FIGURE 12 – *Two particles of TIV, doubly shadowed. The similarity between the shapes of these shadows and those of the model (figure 11) is evident.* (× 55 000)

FIGURE 13 – *Two* T. paludosa *larvae severely infected with TIV. The normal tan colour has changed to a bluish grey.*

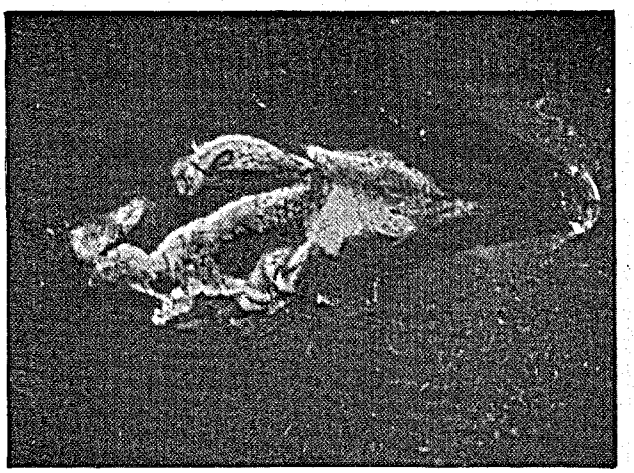

FIGURE 14 – *A* T. paludosa *larva, infected with TIV, dissected to show the blue colour of the hypertrophied fat-body.*

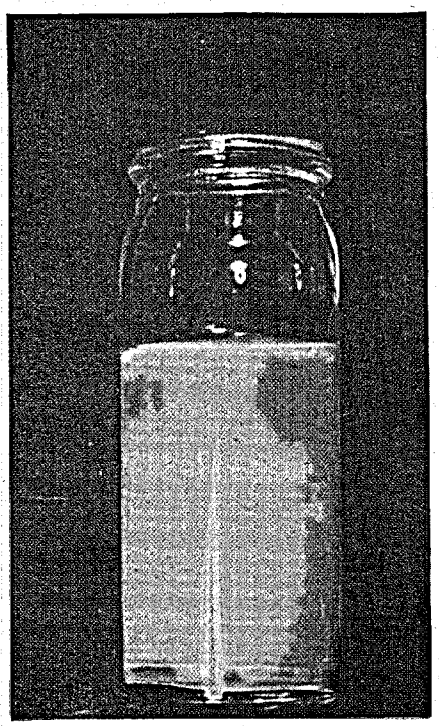

FIGURE 15 – *A bottle of purified TIV that has stood in the cold. Note that a layer of coloured crystallites has formed at the bottom.*

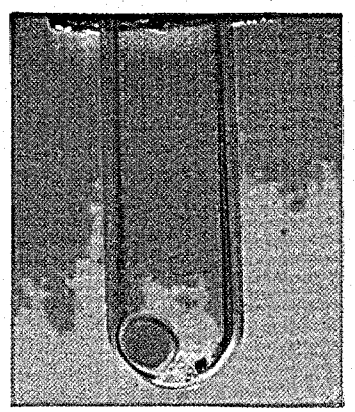

FIGURE 16 – *A centrifuged pellet of purified TIV photographed by transmitted light. The pellet appears uniformly orange.*

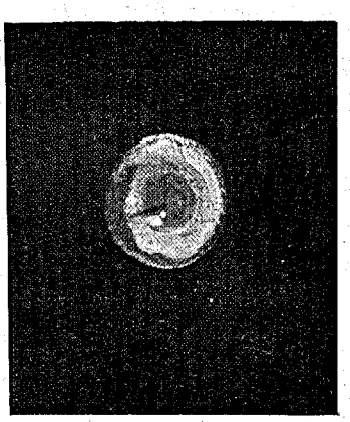

FIGURE 17 – *A pellet of TIV allowed to re-hydrate and photographed by reflected white light. The iridescence is evident.*

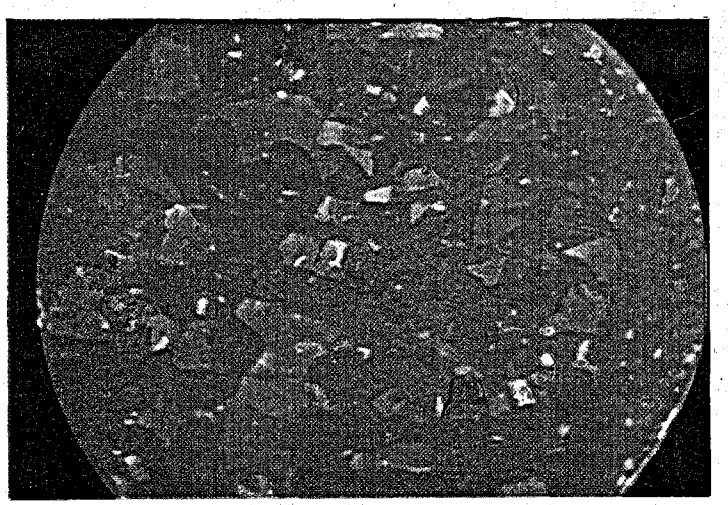

FIGURE 18 – *An array of crystals of TIV photographed by reflected white light.* (× 10)

capsule or granule. No polyhedral bodies are found in cells infected with a granulosis disease. That the encapsulated particles are the causative agents of the granuloses is established from the observation that a suspension of purified granules is highly infectious [10]. When the granules are treated with weak alkali a rather complex structure is disclosed. There is an outer capsule shaped like a prolate spheroid, and inside this there is found a similarly shaped, but separable, capsule (figure 3). Within this inner structure a rod-shaped particle is found, apparently enclosed in a limiting membrane [11]. It is not known if the innermost particle, with its intimate membrane, is infectious.

The granulosis group of viruses has so far been found to be restricted in host range to the caterpillars of Lepidoptera. Examination of thin sections of tissues of infected insects reveals bodies that are similar in shape and size to sectioned granules, and from such observations an indication can be obtained of the likely sites of multiplication of the virus [12]. The sites are chiefly the tissues of the skin and the fat-body. The progress of the disease resembles that induced by the nuclear polyhedroses, in that the body contents are liquefied and are easily liberated by rupture of the skin.

The granulosis viruses may have a practical importance because some of their hosts—like the caterpillars of the white, or cabbage, butterfly—are insects of economic importance. Since a suspension of highly infectious granulosis virus can be produced in large quantity (as much as one gallon from five infected larvae), it is likely that biological control of these lepidopterous pests can be secured through spraying infested fields.

Non-encapsulated viruses. A third group of insect virus diseases is characterized by the occurrence of infectious particles generally similar in appearance to those found in virus diseases of animals and higher plants. The virus particles are not associated with any kind of inclusion crystal or encapsulation, but rather are found free in the cells of the particular tissue attacked. Only two examples [13, 14] of this group are so far definitely known, one occurring in larvae of Diptera, the other in larvae of Lepidoptera. It is possible to extract the particles from the cells and purify them; in both cases the purified, approximately spherical, particles have been found to be infectious. One member of this group of non-encapsulated viruses, here called the *Tipula* iridescent virus (TIV) is of considerable interest, and will be described in some detail later in this article.

HOW INSECT VIRUSES ARE SPREAD

Many of the plant viruses, and some of the viruses affecting higher animals, are transferred from host to host only by means of arthropod vectors, such as aphids, mosquitos, and mites. Insect viruses are mostly, if not wholly, spread from diseased to healthy larvae by the ingestion of infected food. The efficiency of spreading is greatly increased by the way in which the virus particles are, as it were, often 'packaged for delivery', i.e. contained in granules and polyhedral bodies which may retain their infectivity for several years. On the other hand, the particles of the nuclear polyhedral viruses are quite labile after removal from their protein matrix. The manner of development of the nuclear polyhedroses is also favourable towards the spread of disease; the skin of the diseased insect ruptures on death and the liquefied contents of the body get splashed by rain upon plants used for food by healthy larvae. In addition, in some cases the healthy larvae are strongly attracted by the liquefying cadavers, on which they feed with devastating results.

The way in which the cytoplasmic polyhedroses develop, however, is not so favourable to the spread of these diseases. Caterpillars infected with this type of polyhedrosis tend to dry up rather than liquefy, and on death they retain their body structures intact. It is possibly for this reason that these diseases are found to spread more slowly than do the nuclear polyhedroses. The method of spread of the cytoplasmic polyhedra is probably by ingestion of faeces contaminated in the late stages of the disease by polyhedra liberated into the lumen of the gut.

There is very little information about how the viruses that lack inclusion bodies are spread, but presumably they are in some manner ingested with the food. It is a little difficult to visualize how the *Tipula* iridescent virus gets about, since its specific host, the larva of *Tipula paludosa* (the crane fly or daddy-long-legs) is a soil-inhabiting insect. No doubt in this case also the food becomes contaminated, but the comparative rarity of this virus disease may perhaps be accounted for by difficulty in dissemination.

There is good evidence that some of the insect viruses are passed through the egg to succeeding generations. Examples of this type of passage are found in a cytoplasmic polyhedral virus from *Arctia caja* (the garden tiger moth) [15], and in a

granulosis virus affecting the caterpillars of *Pieris brassicae* (the large white butterfly) [16]. This mode of dissemination of disease is of practical importance in some cases where biological control of insect pests is being attempted, since passage of virus through the egg allows us to infect future generations, which is not possible with insecticides. The phenomenon of the passage of a virus to the progeny of infected insects brings us to the consideration of 'latency' in insect viruses.

LATENT VIRUSES

Many examples are known of viruses that may remain dormant in their hosts for prolonged periods, even through successive generations, and that suddenly begin to cause pathological changes in the absence of any overt inoculation of the host. In their dormant stage such viruses are said to be 'latent', an indefinite term indicative of our ignorance of what is happening. Latency is found throughout the virus diseases of bacteria, plants (where it is quite common), the higher animals, and insects. It is encountered with great frequency in the virus diseases of insects, where in some populations almost every individual appears to carry a latent virus. Needless to say, the ubiquitous presence of virus latency among insects renders the precise biological assay of their viruses almost impossible.

It is sometimes possible to cause a latent virus to become virulent by artificial means; such as by rearing under unsuitable environmental conditions and by feeding with chemicals. One of the most successful methods is to introduce into the insect a virus that may in the event prove to be different from the one being carried in the latent form. It has frequently happened during cross-inoculation studies that an insect develops a virus disease different from the one with which it was inoculated. Inoculation with a nuclear polyhedral virus frequently stimulates the development of a cytoplasmic polyhedrosis; but, oddly enough, the reverse seems never to occur. Once a latent virus has been transformed into a virulent one it can be transmitted from insect to insect. This circumstance may have a practical application in some cases: an experience with the larvae of the winter moth, *Operophtera brumata*, provides an example [16]. After a search for a naturally occurring virus affecting this species had failed, some apparently healthy larvae were inoculated with a nuclear polyhedral virus from the caterpillar of a butterfly, *Vanessa cardui*. A very high mortality resulted, all the larvae dying, but they succumbed to a cytoplasmic polyhedrosis. Once this virus had been established in virulent form it was transmitted successfully to numbers of winter moth larvae, allowing a stock of virus suspension to be built up for use in experiments on biological control.

TWO INTERESTING INSECT VIRUSES

Although very few virus diseases have so far been found in the larvae of Diptera (flies), two have such interesting particles associated with them that they merit a detailed description. Both diseases affect the larvae (popularly known as leatherjackets) of *Tipula paludosa*, and both may occur simultaneously in the same insect. One virus causes a polyhedral disease of a type unusual in that the associated polyhedra are not soluble in the usual reagents [17]. Affected larvae exhibit a characteristic pallor that is apparently due to an abnormally large number of blood cells having nuclei largely filled with polyhedral bodies. The virus seems to multiply within the greatly enlarged nucleus of the blood cell; as the disease progresses, the polyhedral bodies begin to form around the periphery of the nucleus in close proximity to the nuclear membrane (figure 4).

As has been mentioned earlier, nuclear polyhedra usually dissolve readily in weak alkali, leaving behind a thin membrane containing a number of virus rods. The behaviour of the nuclear polyhedra of *T. paludosa* towards different reagents is unusual, however, and differentiates them sharply from the other known types of nuclear polyhedra. They are resistant to trypsin, and to dilute and weak acids and alkalis. In molar sodium hydroxide, however, they elongate to several times their normal length, becoming first biconvex spindles and then extending into crescents or worm-like shapes. At about three times their normal length the elongation is still completely reversible, and in water at *p*H 5–8 they return to their original size and shape. The elongation and the return to normal length (which takes place along the same axis) can be repeated indefinitely and as rapidly as the necessary solutions can be alternated. The failure of the polyhedral bodies to dissolve makes it impossible to see by electron microscopy any virus particles contained within. It is necessary to examine thin sections of the polyhedra in order to see what is inside them. In the electron microscope the sections reveal the presence of rod-shaped particles, presumed to be the elementary virus particles. Figure 5 shows some such sections, some apparently longitudinal and some transverse.

Larvae of *T. paludosa* collected from the field are occasionally found to be harbouring a virus disease easily recognizable by the change in appearance that it induces in the body of the affected host [14, 18, 19]. Whereas the normal appearance of these larvae is a dark tan, the colour of the diseased ones is a somewhat opalescent blue-indigo (figure 13). Examination by low-power microscopy shows that the colour is particularly intense in the lobes of the hypertrophied fat-body (figure 14); as the disease approaches its termination the colour is less intense and is more generally dispersed throughout the body. When sections of diseased fat-body tissue are examined in the electron microscope it is seen that the cells are filled, but only in the cytoplasm, with darkly staining particles (figure 6), believed to be the elementary virus particles. There is no indication of encapsulation around the virus particles, nor is there any sign of the development of a polyhedral body, such as would be seen in a cytoplasmic polyhedrosis.

It is quite easy to extract and purify the elementary particles from the diseased insects. Immediately after death has ensued the cadavers are simply placed in water, where they are allowed to stay for a few days while the particles leach out. A brief, low-speed centrifuging is sufficient to remove the gross material, after which a high-speed centrifuging will convert the virus particles into a pellet of fairly pure material. A repetition of the low- and the high-speed centrifuging will result in a preparation that is demonstrably homogeneous and is presumably pure. When this material is fed to healthy larvae there is an incidence of the disease distinctly higher than that found in the wild population: from this observation it may be concluded that the particles in the preparation are the causative agent of the disease. The amount of virus produced by one larva is remarkably great: measurements so far made indicate that in the late stage of the disease about one-fourth of the larva's dry body-weight will have been converted to virus particles. This figure is not approached by any other known animal virus disease, although it is approximated by the tobacco mosaic virus disease and by a few of the virus diseases of bacteria.

Pellets formed from purified TIV are found to have fascinating optical properties. When observed by transmitted light they are generally orange in colour (figure 16), not greatly different from the colour exhibited by pellets of other viruses. But when examined by reflected white light the pellets appear exquisitely iridescent, with the colours most noticeably in the blue and green regions of the spectrum. The predominant colour is related to the degree of hydration of the pellet; very 'wet' pellets are more yellow and orange (figure 17), while 'dry' ones are characteristically violet. The origin of the colour is disclosed by examination of thin sections cut through pellets that have been dehydrated and embedded for sectioning. A typical section will show a somewhat bizarre pattern of particle array, such as is shown in figure 7. As is readily seen, this pattern is what would be expected if a thin slice were made through an array of randomly orientated microcrystals. From this kind of observation it is concluded that the pellet is a mass of small crystals, each crystal being of the order of 10 microns across. In fact, the composition of a pellet can be thought of as a scaled-up model of a polycrystalline metal, such as a piece of cast alloy. It is well known that a specimen of a polycrystalline metal will exhibit Bragg reflections when illuminated with X-rays. The conditions for such reflections are that the interatomic spacings in the crystals be about half the wavelength of the X-rays employed, and that the orientation of a crystal with respect to the incident X-ray beam be such that the scattered, or 'reflected', X-rays experience constructive interference. If we were to build our crystal from large-size virus particles instead of from atoms, and substitute light waves for X-rays, we should have the proper situation for the exhibition of Bragg reflections in the visible region of the spectrum. Since we illuminate with light of all wavelengths (white light), each virus crystal will be oriented in a manner such as to reflect constructively some particular wavelength, and the overall effect will be iridescence—the reflection of light of differing colours by multiple, tiny regions. Pellets exhibiting these optical properties have not been seen for any other virus, owing to failure to satisfy the condition that the optical path-length between adjacent planes in the crystals be as large as half the wavelength of visible light. But the particles of TIV are so large that the inter-particle spacing is about 1500 Å, and, assuming a refractive index of about 1·5, the optical path-length is about 2300 Å. Hence constructive interference should occur with light of blue-green colour. Other animal viruses are known that are even larger than TIV, but they are apparently not sufficiently uniform in size and shape to crystallize.

Purified preparations of TIV have a tendency to crystallize when allowed to remain undisturbed

in the cold, although their behaviour in this respect is not entirely predictable. A suspension of TIV in an ordinary glass tube will generally be found to have crystallized in part, with a mass of small, brilliantly reflecting crystals at the bottom and a diminished concentration of virus in the supernatant fluid (figure 15). On one occasion it was possible to form an array of crystals on the flat surface of a microscope slide, in which position they can readily be photographed (figure 18). Attempts made so far to induce crystallization by the addition of half-saturated ammonium sulphate (the classical method of crystallizing plant viruses) have been only moderately successful. Since it would be of considerable interest to examine such crystals by X-ray and optical analysis, the conditions necessary for their formation, and their stability when formed, must be further investigated.

Since the particles of TIV may be obtained obviously quite pure and in considerable quantity, they are fit subjects for chemical and physical investigations designed to give a better understanding of the structure of viruses. Only preliminary chemical analysis has so far been undertaken, and from this it appears that the virus particles consist of nucleoprotein. The nucleic acid portion is all of the deoxy type and constitutes about 15 per cent of the mass of the virus. A determination of the proportions of basic substances indicates that these are distributed much as in other insect viruses analysed. Preliminary sedimentation studies show that the virus particles form a sediment with a sharp boundary—a result not surprising in view of the high degree of morphological homogeneity, indicated by the evidence of crystallization and by electron microscopy.

The morphology of the particles of TIV may be examined by electron microscopy of thin sections of material containing them and of preparations of purified virus. Micrographs of sections show two characteristic features (figure 8), one expected and one new. The expected observation is that the particles possess to some degree a non-uniform internal structure. There appears to be an outer envelope, within which is a relatively transparent region; the central area of the particle is filled with opaque material, tentatively identified with its nucleic acid portion. Such differentiation of apparent structure is commonly encountered in sections of the larger animal viruses. The unexpected observation is that six-sided contours fairly frequently occur when the virus particles are seen in section. Regular hexagons are not seen, presumably because of distortion of the sectioned material by the microtome knife, but the relation of the direction of the apparent distortion to the known direction of cutting is such as to imply that the true contours are hexagonal.

Purified preparations of TIV appear quite monodisperse in the electron microscope, each virus particle having a diameter of about 1300 Å. Even in dry specimens prepared from a water suspension in the usual way, and hence suffering the distortions brought about by surface tension, the characteristic contour of the particles is six-sided (figure 9) rather than circular. This appearance is unique among the known viruses of comparable size: vaccinia, for example, is brick-shaped and non-uniform in size, while influenza appears quite circular and heterodisperse.

Since the particles of TIV appear to have six sides when seen in contour, it is reasonable to suppose that they are polyhedral in shape. Other examples of polyhedral-shaped virus particles are known [20] among the bacterial and plant viruses. But in no case has it hitherto been possible to arrive at a convincing notion of the exact form of the polyhedron. The difficulty has been mainly due to the smallness of the particles, making a direct determination of the shapes of the polyhedral facets uncertain. An indirect approach is to infer the three-dimensional shape of polyhedra from the shapes of the shadows formed by application of a shadow-casting technique [21]. With the small viruses this method is unreliable, owing to the relative roughness of the surface of the substrate film (cellulose nitrate) upon which the virus particles must be mounted [22].

The particles of TIV are large enough, and their shape is regular enough, to offer some hope that their full polyhedral shape can be determined by the analysis of shadow shapes. A direct observation of the shapes of the surface facets cannot be undertaken, owing to the great electron opacity of such large objects. In order to attempt shadow analysis it is essential to preserve the three-dimensional shape of the virus particles as they are dried for electron microscopy. This preservation is readily and effectively secured by freeze-drying [23]. As can be seen (figure 10), shadows of particles so prepared are distinctive in shape, and their outlines are not obscured by roughness of the substrate film.

Only three kinds of polyhedra will have six-sided contours when the object is lying upon any one of its facets. These are the octahedron, the rhombic dodecahedron, and the icosahedron. But

as can be seen by trials with models, the octahedron will not cast a shadow having five sides, a type of shadow frequently cast by the frozen-dried particles of TIV (figure 10). The decision, then, lies in a choice between the rhombic dodecahedron and the icosahedron. The correct answer may be found by the application of double shadowing, in which two shadows are cast with azimuth angles 60° apart. In this way a single particle can, as it were, be examined from two points of view. A cardboard model of an icosahedron, illuminated by two light sources placed 60° apart relative to the model, is shown in figure 11. These points are to be noted: when the orientation of the model is such that one point of the hexagonal contour is directed toward one shadow, and the adjacent point is directed toward the other shadow, one of the two shadows formed will be five-sided and with a blunt end, while the other is four-sided and is pointed. A rhombic dodecahedron will not cast a pair of similarly shaped shadows when similarly illuminated. In figure 12 are shown two frozen-dried particles of TIV that have been double-shadowed to test the aptness of the icosahedral model. As can be seen, the correlation in shadow shapes is good. We can therefore conclude that the three-dimensional morphology of one virus is now known and that its shape is that of an icosahedron. What bearing this determination will have upon furthering our notions of virus structure remains to be seen.

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