Part 2. Biotechnology

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TRISYMMETRONS AND TUBULAR FORMS OF IRIDOVIRUS FROM THE MOSQUITO *AEDES* (*OCHLEROTATUS*) *CANTANS* (MEIGEN, 1818) (DIPTERA: CULICIDAE)

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Summary. Mosquito iridescent viruses (MIV) were isolated in Ukraine from the larvae of the bloodsucking mosquito *Aedes* (*Ochlerotatus*) *cantans* (Meigen, 1818) (Diptera: Culicidae) that were infected. Examination of ultrathin sections of infected mosquito fat body cells revealed that MIV virion maturation occurs in the cytoplasm. The virions were spherical with pentagonal and hexagonal outlines, indicating icosahedral symmetry. In addition to spherical virions with a diameter of 190 ± 5 nm, smaller tubular structures were present in the cytoplasm of infected cells. Upon destroying the purified virus, a large number of trisymmetrons were identified. Each trisymmetron consists of 55 hexagonally arranged subunits that form an isosceles triangle with an edge length of 60 nm

Keywords: MIV, IIV-3, electron microscopy, morphology, mosquito larvae

Introduction. Mosquito iridescent virus (MIV, or IIV-3) is a member of the *Chloriridovirus* genus, which is part of the Iridoviridae family. This family includes large (120–350 nm) DNA-containing spherical viruses from many invertebrates (beetles, butterflies, mosquitoes, midges, ticks, bees, isopods, crabs, oysters, spiders, scorpions, nematodes) and vertebrates (fish, amphibians, reptiles). The name of the iridovirus family comes from the word 'iris', which means 'goddess of the rainbow' in Greek. The name of the genus *Chloriridovirus* comes from the word chloros, which means 'green' in Greek.

For the first time, the mosquito iridescent virus of the *Ochlerotatus* subgenus was detected almost simultaneously in 1965 in the Czech Republic in the larvae of *Aedes* (*Ochlerotatus*) *annulipes* (Meigen, 1830) and *Aedes* (*Ochlerotatus*) *cantans* (Meigen, 1818) (Weiser, 1965) and in the USA in the larvae of the black salt marsh mosquito, *Aedes* (*Ochlerotatus*) *taeniorhychus* (Wiedemann, 1821) (Clark, Kellen and Lum, 1965). It was then isolated from many species of this subgenus in various countries of Europe, Asia, North America, and South America, including Ukraine (Buchatskiy and Sheremet, 1974; Buchatskyi, Kaniuka and Lebedinets, 1976).

Because of the large size and complex structure of MIV spherical virions, many details about their structure and the mechanism of virion maturation in infected cells remain unresolved. In this work, the tubular forms and trisymmetrons of the iridovirus from the mosquito *Ae. cantans* are described for the first time using the method of electron microscopy.

Aim of this research was to study the ultrastructure of purified mosquito iridovirus virions during their degradation during long-term storage in distilled water and to perform electron microscopic analysis of ultrathin sections of fat body cells of *Ae. cantans* mosquito larvae infected with iridovirus.

Materials and methods. Mosquito iridescent virus was isolated from fourth-instar larvae of mosquitoes *Ae. cantans*, which were found in one of the reservoirs in Kyiv Region. The virus was cultivated in larvae of the honeycomb moth, *Galleria mellonella* (Linnaeus, 1758) (Lepidoptera: Pyralidae) and cell cultures of *Ae. aegypti* (Mos 20A) as described earlier (Buchatskyi, Victorov-Nabokov and Sheremet, 1976; Sutugina et al., 1995).

MIV was purified by differential centrifugation and subsequent centrifugation in a sucrose density gradient (10–50%) for 40 min in a Beckman L5-50B ultracentrifuge in a SW-40 rotor (Buchatskiy and Sherban, 1976). To obtain trisymmetrons, the purified mosquito iridovirus was stored for two months in distilled water at a temperature of 4°C.

For electron microscopy, purified MIVs were negatively stained with 2% phosphotungstic acid (PTA) adjusted to pH 6.8 with 1 M KOH. For thin layer electron microscopy, infected larvae were fixed in phosphate-buffered 2.5% glutaraldehyde, post-fixed in 1% osmium tetroxide, dehydrated in graded alcohols, and embedded in 812 Epon resin. Ultra-thin sections were double-stained in uranyl acetate and lead citrate. Examinations were performed with JEMB-100B electron microscope.

Results. After long-term storage in distilled water, purified MIV virions disintegrated into separate components. As for other iridoviruses of insects and fish, the most numerous components of their degradation were trisymmetrons (Fig. 1). Each of them consisted of 55 hexagonally arranged subunits, forming an isosceles triangle with a rib length of 60 nm. Ten protein subunits

were placed on each rib. MIV trisymmetrons were often located close to each other and had the same number of such subunits (Fig. 1). In contrast, pentasymmetrons, which are another component of icosahedrons, did not have a clear image of capsomers, because they are three-dimensional structures and their capsomers overlap each other in electron micrographs. It is known from crystallographic conditions that an icosahedron with triangulation number T = 147, including MIV, can be built from 20 trisymmetrons and 12 pentasymmetrons (Caspar and Klug, 1962; Stoltz, 1971, 1973; Parvez, 2020).

As in other insect iridoviruses (Wrigley, 1969, 1970), each pentasymmetron contains thirty-one protein subunits. Therefore, the total number of capsomeres in the MIV can be calculated by the formula: N=(55×20)+(31×12)=1,472.

Examination of ultrathin sections of infected mosquito fat body cells showed that maturation of MIV virions occurs in the cytoplasm. Virions had a spherical shape, pentagonal and hexagonal outlines, indicating an icosahedral type of symmetry. Next to the mature forms of virions with a diameter of 195 ± 5 nm in the cytoplasm there were a large number of immature virions that were at various stages of the formation of the viral capsid and tubular forms of virions (Fig. 2). The diameter of these tubular forms was much smaller than that of virions, ranging from 110 to 165 nm. Their length could reach up to 1.2 μm (Fig. 3). One of the ends of the tubular form of the virus was often closely connected with the incomplete form of the virion (Figs 4–6), which may testify in favor of the hypothesis of the formation of the spherical capsid of the virus from tubular forms. On ultrathin sections of infected cells, such tubular forms of the virus were often located near mitochondria (Fig. 7).

Discussion. The presence of trisymmetrons among the destroyed virions of large spherical DNA-containing viruses and the phylogenetic relationships between them has been described in many works (Wrigley, 1969; Xiao and Rossmann, 2011; Yutin and Koonin, 2012; Zhao et al., 2023). In terms of morphology, trisymmetrons of other viruses did not differ from those described by us, except for the length of the ribs. For the iridovirus of the European crane fly, *Tipula paludosa* Meigen, 1830 (Diptera: Tipulidae), the length of the ribs of the trisymmetrons was 70 nm (Wrigley, 1970; Manyakov, 1977), and for MIV it is 60 nm, that is, less.

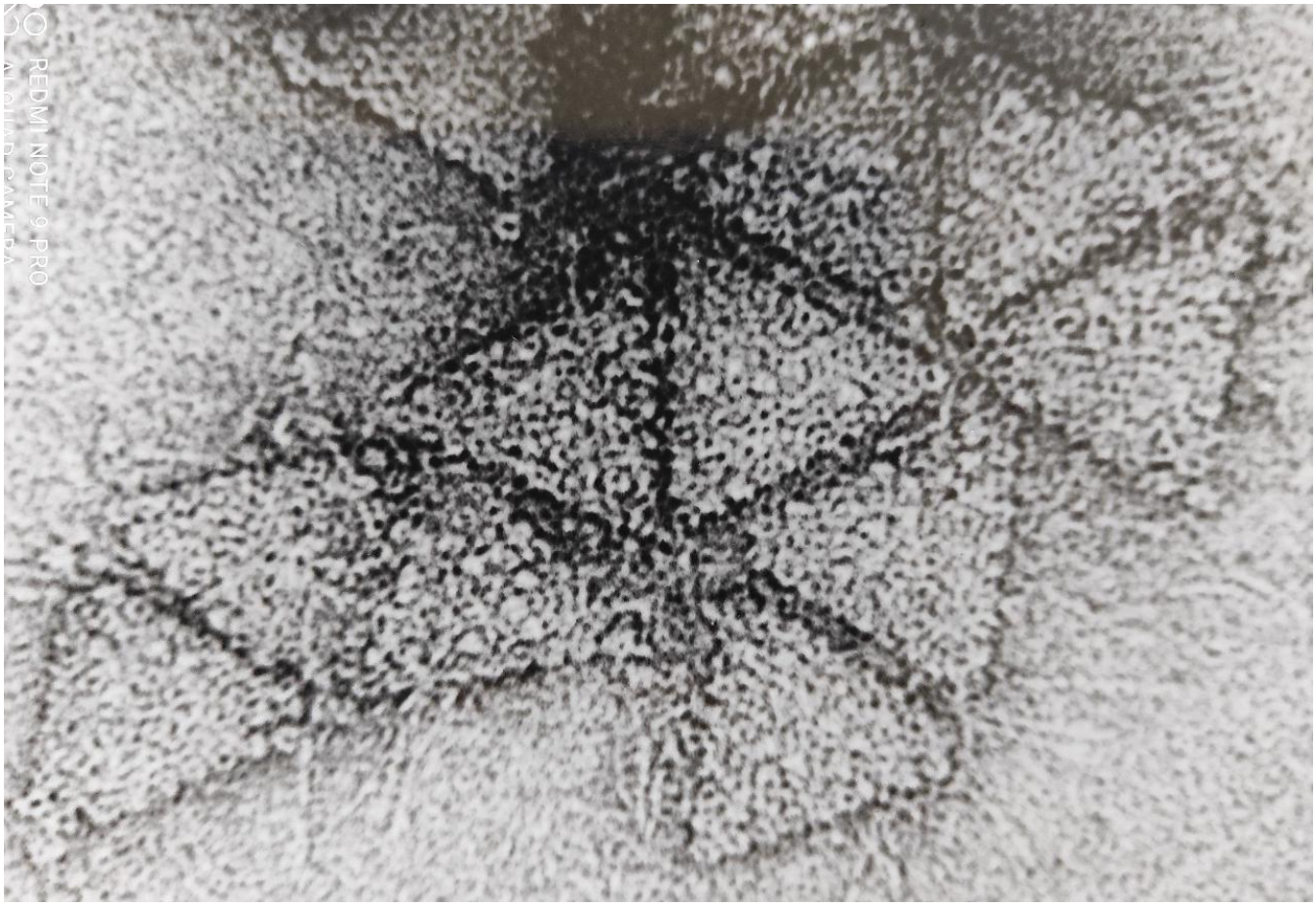

Figure 1. Trissymetrons of the iridovirus from the mosquito *Ae. cantans.* The length of the ribs is 60 ± 2 nm.

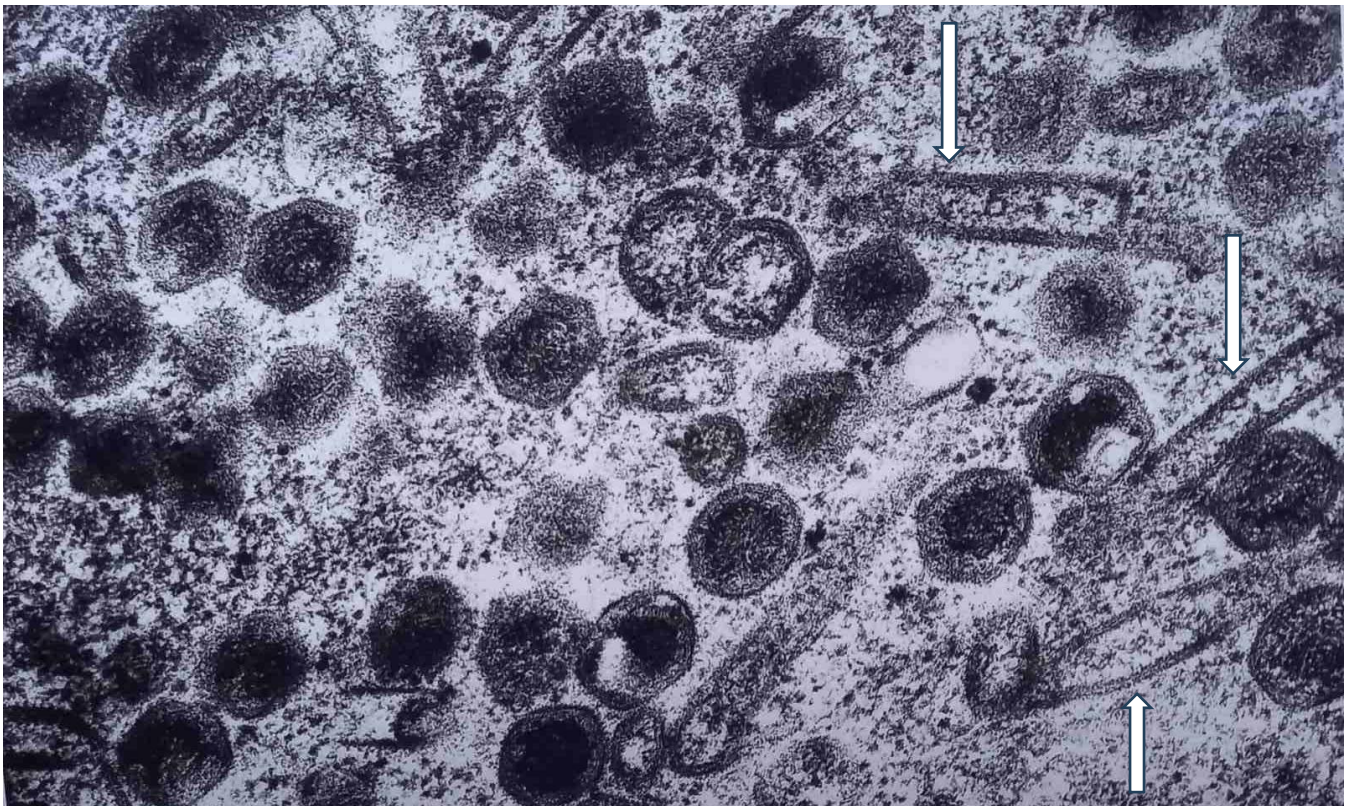

Figure 2. Tubular forms (indicated by arrows) of *Ae. cantans* iridovirus among mature and immature forms of the virus. Diameter of virions is 195 ± 5 nm.

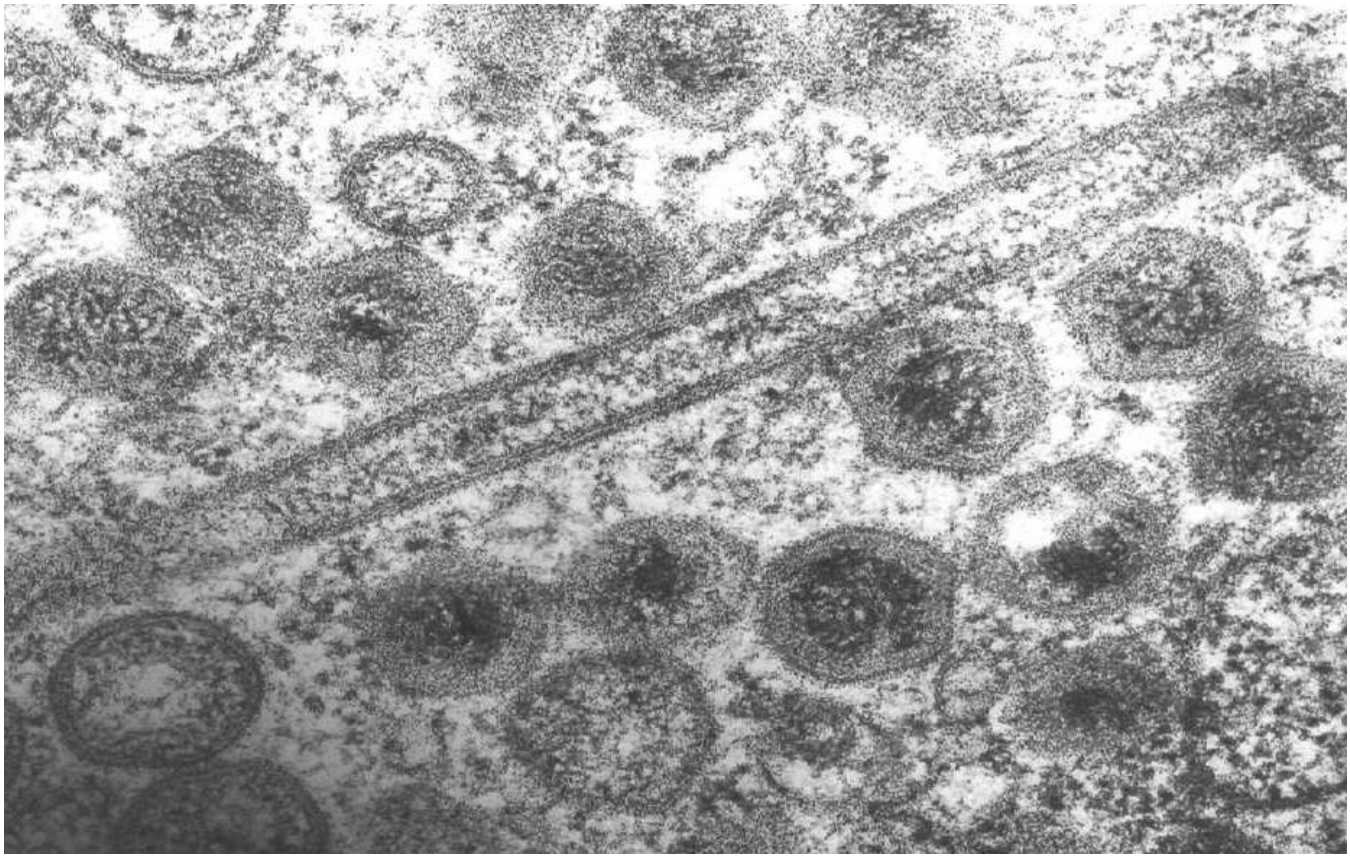

Figure 3. The tubular form of the iridovirus from the mosquito *Ae. cantans* is more than 1 μm in length. Diameter of virions is 195 ± 5 nm.

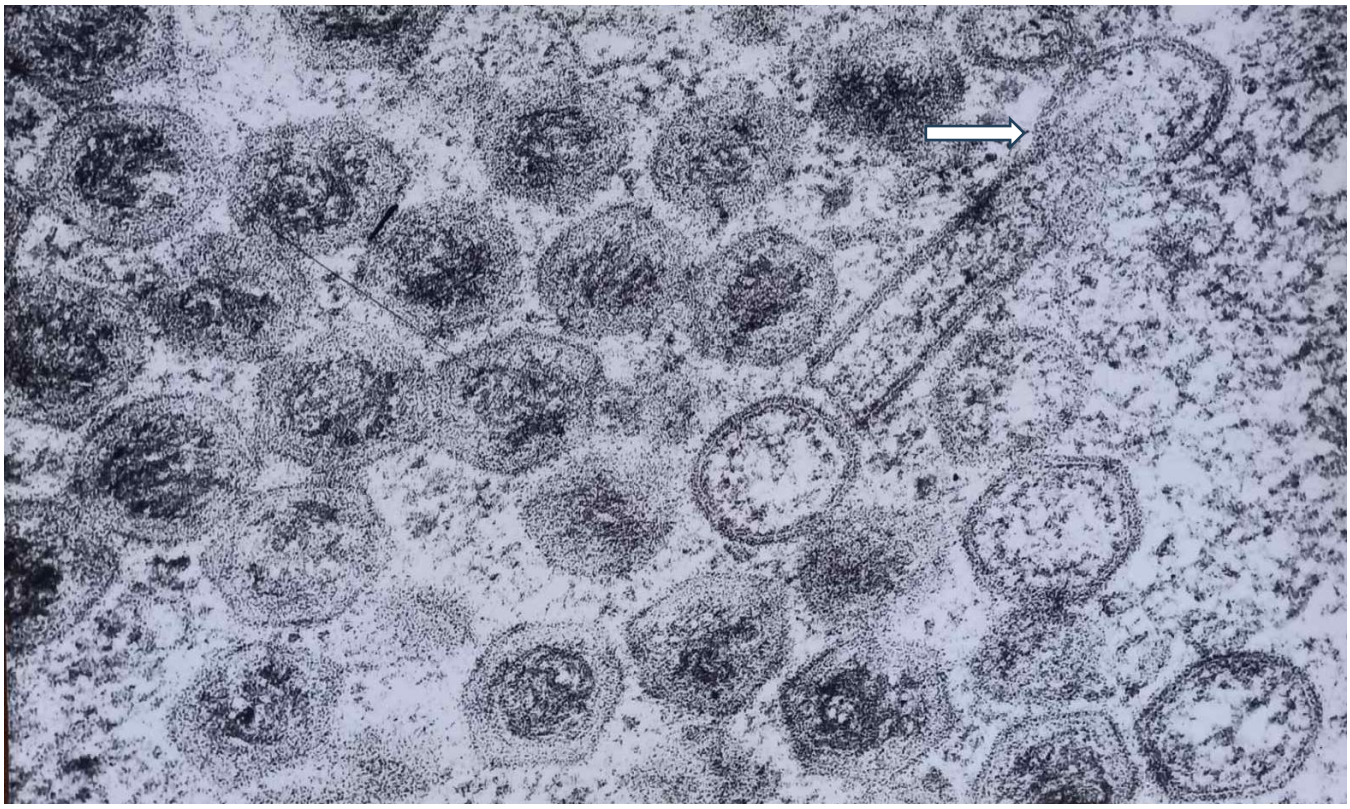

Figure 4. The tubular form of the iridovirus of the mosquito *Ae. cantans*, at one end of which a viral capsid is formed. Diameter of virions is 195 ± 5 nm.

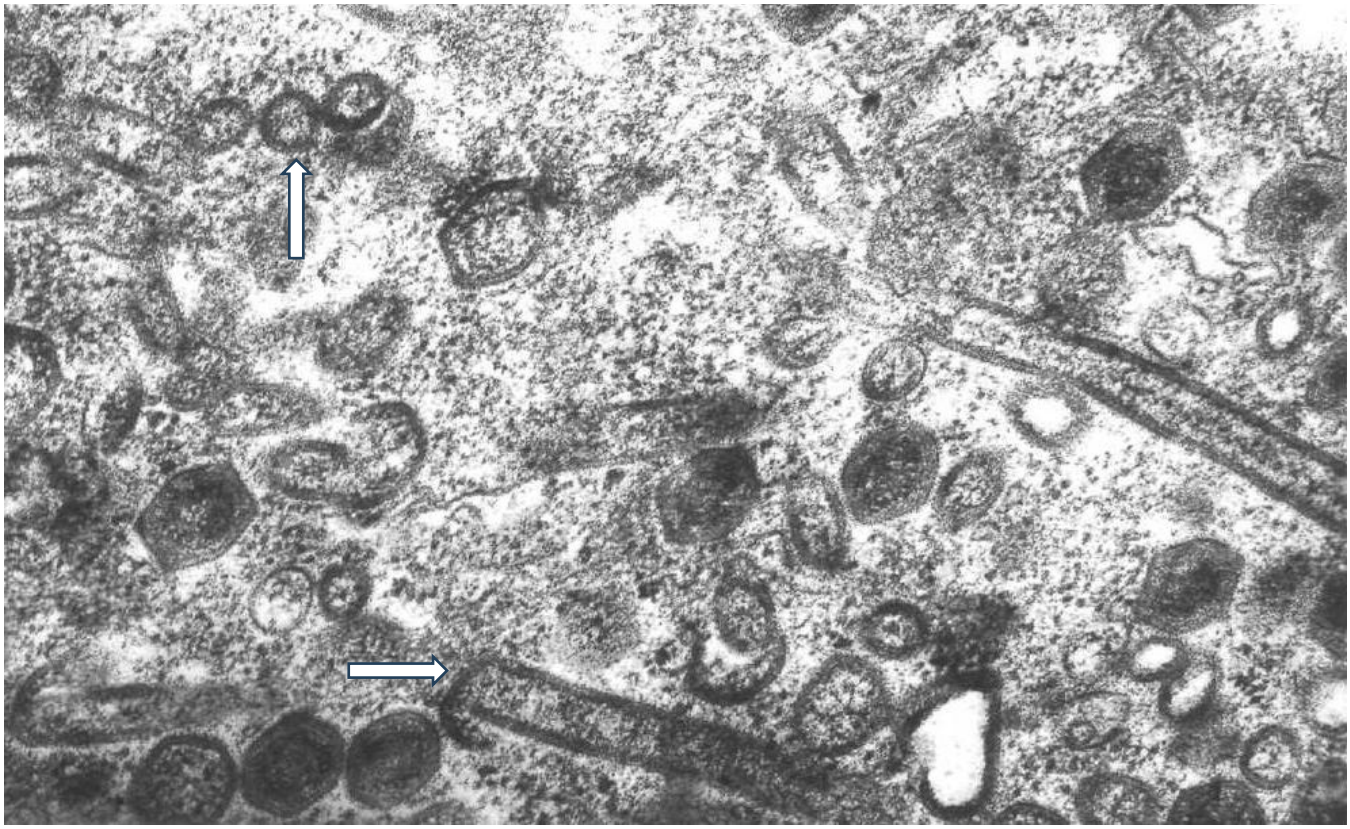

Figure 5. Tubular forms of iridovirus of the mosquito *Ae. cantans.* A viral capsid is formed at one end of the tubular form (horizontal arrow). A cross-section of the tubular form of the MIV is marked with a horizontal arrow. Diameter of virions is 195 ± 5 nm.

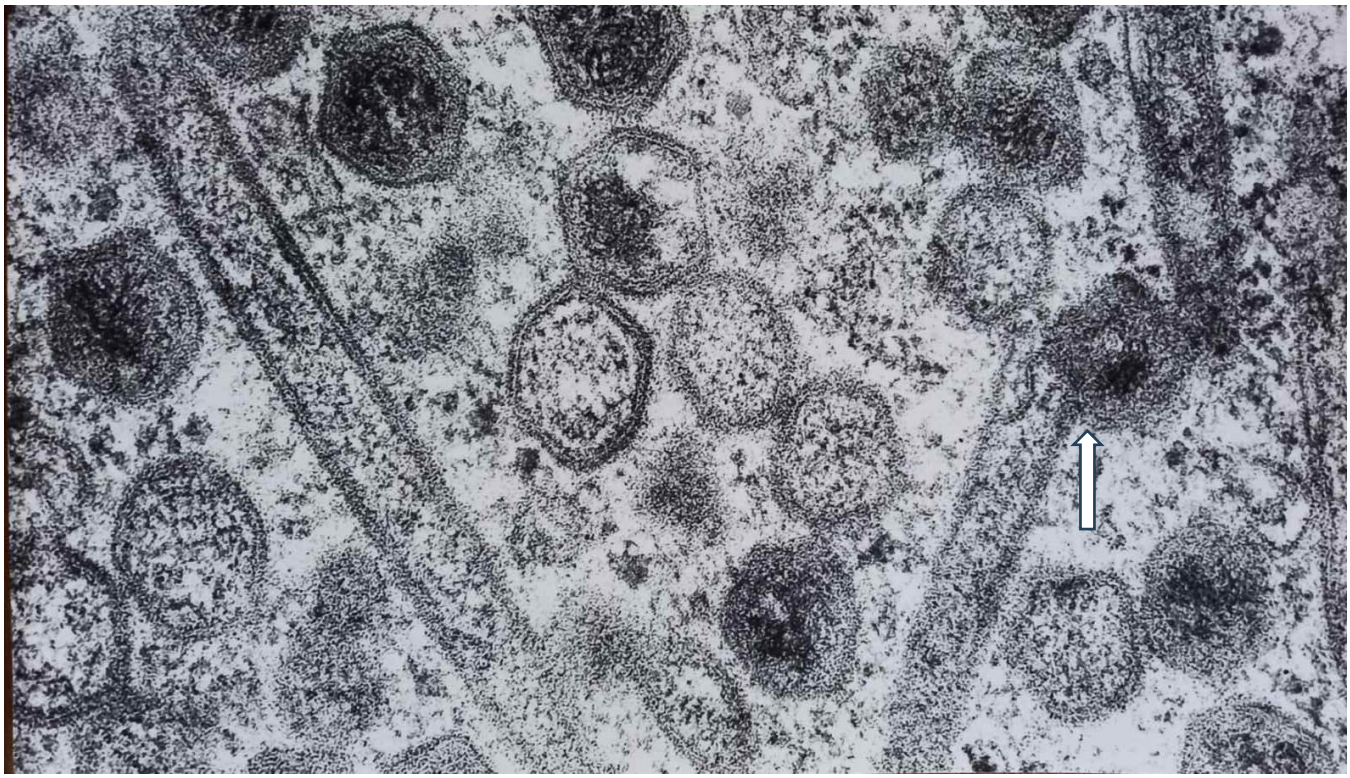

Figure 6. Tubular forms of iridovirus of the mosquito *Ae. cantans*. At one of the ends of the tubular form, the viral capsid is formed (arrow). Diameter of virions is 195 ± 5 nm.

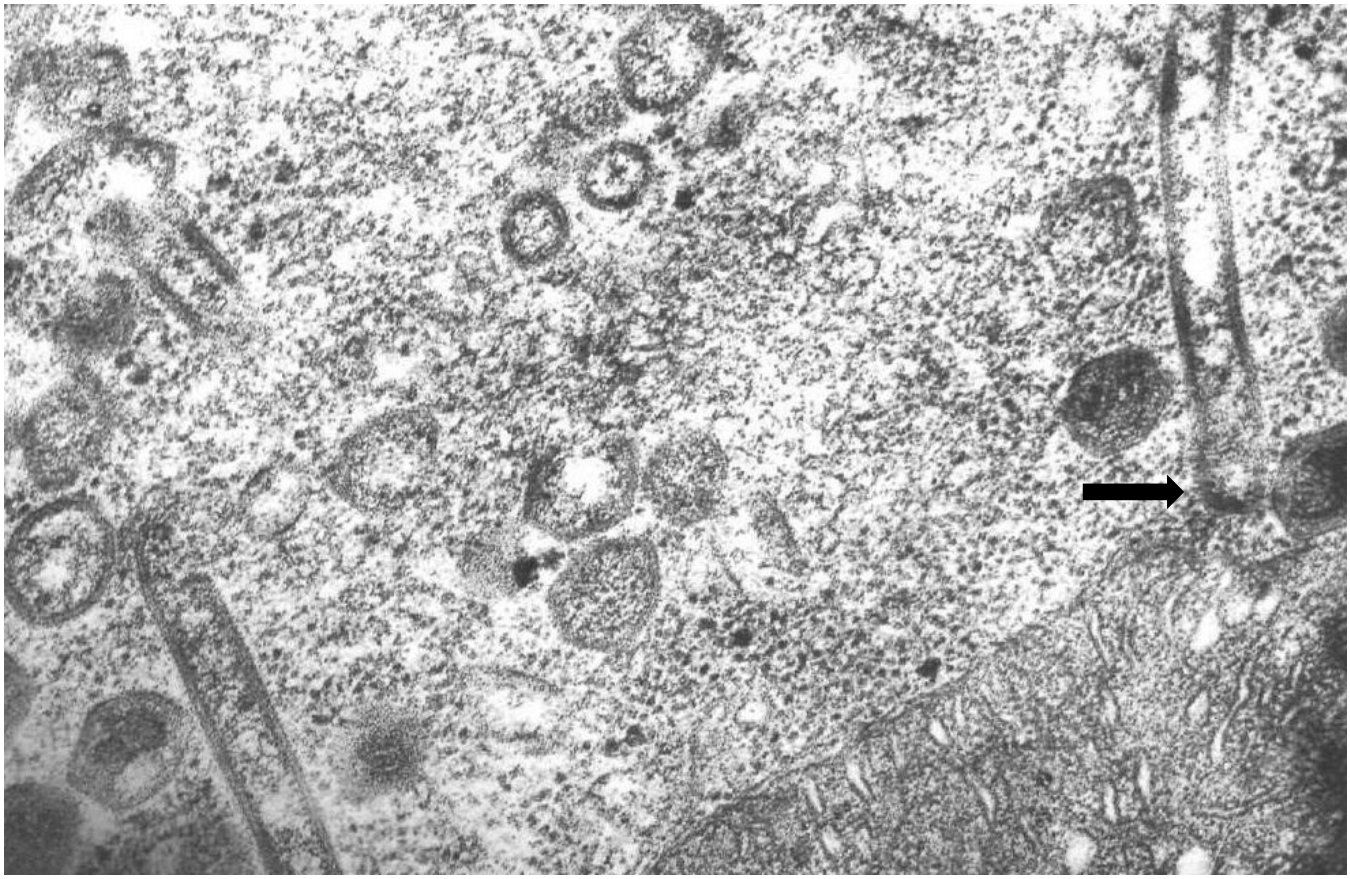

Figure 7. The tubular form of the iridovirus of the mosquito *Ae. cantans* is found near the mitochondria with broken cristae. Diameter of virions is 195 ± 5 nm.

The presence of tubular forms of viruses in infected cells has been established for many viruses. They have been described both for plant viruses (Bancroft, Hills and Markham, 1967; Hitchborn and Hills, 1967; Chen et al., 2012) and for animal and human viruses such as African swine fever virus (Epifano et al., 2006), foot-and-mouth disease virus (Ghosh, Borca and Roy, 2002), bovine reovirus (Kimura and Hase, 1987), herpes virus (Iwasaka, Mori and Oda, 1980), alphavirus (Kril et al., 2024), papovaviruses (Arnold, 1979; Keef, Taormina and Twarock, 2005), hepatitis B virus (Neurath et al., 1976), human immunodeficiency virus HIV-1 (Bharat et al., 2014) and other viruses. Until now, the mechanisms of maturation of iridoviruses have not been fully studied. There are two alternative views on this: (1) an envelope forms around a pool of DNA; (2) first, the envelope is formed, then DNA penetrates through the unfinished capsid. However, it is difficult to decide in favor of one of these two mechanisms of virion maturation of iridoviruses. Considering the presence of a large number of enzymes in the composition of iridoviruses (Eaton, Ring and Brunetti, 2010), it can be assumed that both points of view are valid, that is, these two processes can go simultaneously. We also assume that one of the alternative ways of forming the capsid of MIV virions is their 'slicing' from the pool of viral proteins existing in the form of tubes.

We found such tubular forms of MIV not only in mosquitoes *Ae. cantans*, but in other mosquito larvae infected with this virus — *Aedes* (*Ochlerotatus*) *dorsalis* (Meigen, 1830), *Aedes* (*Ochlerotatus*) *caspius* (Pallas, 1771), which indicates their universal importance.

Conclusions. During the degradation of MIV, its capsid breaks up into trisymmetrons with 60 nm edge lengths. Each edge contains ten protein capsomeres. There are 1,472 capsomeres in a virion. Tubular forms of the iridovirus were detected in the fat body cells of infected *Aedes* (*Ochlerotatus*) *cantans* mosquito larvae.

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