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A POX-LIKE VIRUS IN THE MIDGE *CAMPTOCHIRONOMUS TENTANS*

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Summary. — A new virus with oval protein inclusion bodies in the cytoplasm of the fat body cells of *Camptochironomus tentans* is described. The virus particles are flat cushion-like bodies, distributed in the protein mass. The inclusion bodies are formed on the surface of the nuclei and the internal structures of the nuclei of infected cells are changed.

Introduction

A preliminary report on the infection under consideration (Weiser, 1948) was published at a time when I had no way to prove the viral aetiology of the infection except infection experiments and analogy with staining qualities of typical polyhedra. Steinhaus (1949) included the description of this infection in his textbook and it was my intention to prove the viral aetiology of the infection by ultrathin sectioning of the material. After 20 years there was no more fresh material available, but regenerated paraffin material was used for this study intended as an amendenent of the early papers on this infection.

Materials and Methods

The material was collected in 1942 in an eurotrophic lake, Drecksee, near Plön, Germany, in bottom samples from the depth of 11 m. A large population of last instar larvae of *Camptochironomus tentans* was washed from the samples, many of them with white chalky cysts in the fat body. The cysts were masses of spores of *Plistophora chironomi*, in other animals *Rickettsiella chironomi* and in a part of the larvae there were masses of oval inclusion bodies in the cytoplasm of fat body cells. The different infections have been described (Weiser, 1943, 1948, 1949).

Insects with white cysts, separated according to the type of infection, were reared in the laboratory in tap water on flat dishes and the infection was studied. From the first symptoms of "whitening" the disease lasted from 7 to 14 days when at 10 to 15° C. At the end of this period, the larvae ceased the vavy motions necessary for respiration and died. The fat body, first segmental, changed to long continuous lobes and disintegrated at the time of the death. The liberated inclusions were distributed during the last hours in the haemolymph and sedimented in the lower parts of the body.

Living larvae were fixed with Bouin's fluid and with sublimate-alcohol and embedded into paraffin for sectioning. Sections were stained with Heidenhain's iron haematoxylin, with Mallory's stain and some slides were stained after Komárek and Breindl (1924) in Giemsa's stain with 0.1% of Na_2CO_3. When stained overnight, the "virus agglomerations" in the interior of virus polyhedra stained red.

After 26 years the material from paraffin blocks and from section preparations was dewaxed and refixed as indicated by Weiser and Žižka (1968); ultrathin sections were stained with uranyl acetate and lead citrate for study in the electron microscope, to demonstrate the viral aetiology of the infection.

Results

Light microscopy

The visible infection was distributed only in the fat body; in some cases the lymphocytes collected some inclusions from bursted cells. The most apparent change in the fat body was a multiplication of the nuclei which were about twice as numerous as in normal fat body lobes (Weiser, 1948, 1949). They were smaller than normal, mostly elongated, triangular, in cross section with an obvious nucleolus. An essential part (about 1/3 to 1/4) of the nuclei were changed at the moment of fixation in connection with the developing infection. It seems to have been the rule that during the prodromal phase of the infection the cell nucleus divided twice. Two equal nuclei resulted of the first division. But only one of them (the healthy one?) produced a second couple of daughter nuclei. The other (statical) nucleus did not divide and in its interior a dark zone filling the whole nucleus was produced. There was no increase in size of the nucleus. Close adherent to the dense nucleus, one or several proteinic inclusions of the virus were produced. On the contact zone, the nucleus fitted its surface closely to the oval, spherical or tetrahedral shape of the inclusion and in many cases, when several inclusions were formed in the same cell, the nucleus formed a connective central mass wherein all inclusions were embedded by a part of their surface (Fig. 1).

As they grew, the necessity occurred to adapt their shape to the space available and so facets present in the inclusions occurred. At the time of the first growth of the inclusions, the fat droplets in the fat body cells disappeared and the whole free space was taken by the proteinic inclusions. Their size varied from 2 μ to 10—16 by 8—10 μ. In the interior of the inclusion bodies many minute granules, more or less refringent depending on the preparation, very prominent after hydrolysis with 0.1 N HCl, appeared under a homogeneous surface layer. All inclusions, minute or big, spherical, oval or tetragonal, bore the granules. The same as whole inclusions without hydrolysis shone white in dark field, mainly the internal granules were refringent in dark field after hydrolysis. These inclusions stained red with alkaline Giemsa stain.

Electron microscopy

The former history of the material did not allow to study the detailed conditions of formation of inclusion bodies in infected cells and only the content of the proteinic inclusions could be demonstrated on ultrathin sections. These structures have been well preserved in some protein masses, in others the virus particles were more or less liberated in vacuoles and in sections in many instances free virus particles were present in the cytoplasm of the cells.

Young stages deposited in groups in the cytoplasm (Fig. 2A, B) were oval membranes with an irregular granulation in the interior. They usually measured 300 by 225 nm, the wall was 30—40 nm wide. During further development, a dense structure was formed in the interior and the general oval shape changed to a cushion-like flat particle with the dimensions of

200—250 × 270—300 × 130—150 nm and rounded corners and edges. The covering layer was homogeneous in well conserved particles in the polyhedra, 15 to 25 nm in cross section. The internal structure was a flat central body of two layers sandwiched together with connected edges (Fig. 3C). The central hole was filled with a dense substance. When liberated from the protein substance, the particles were inflated, oval to subspherical. The covering layer was two-layered, the external 6 and the internal 7 nm thick. The central body was a biconcave mass with a covering dense layer 19—20 nm in cross section and a foamy pulp (Fig. 3D).

Virus particles were distributed in the protein mass at distances equal to the length of the particles and several hundreds of particles were present in one protein inclusion body. The zone seen in the optical microscope after alkali-treatment as an empty covering layer (Fig. 1) was not empty, it contained the same density of virus particles as in the other, internal zone. The effect may have been produced by different densities of the protein in the two zones of the inclusion body.

Discussion

The virus described in this paper has no analogy in former descriptions of viruses occurring in insects, but it seems to be very close to the Vagoiaviruses in its localization in the host, the formation of inclusion bodies and the infection produced. Therefore a comparison of both may be useful.

Infected tissue. In both infections, only the fat body is the site of the development of the virus in its typical form, the inclusions. In both, the masses of inclusions cause hardening of infected tissues and no dissolution, autolysis, of the tissues. The seat of the infection is the cytoplasm of the fat cells. In Vagoiavirus there is no evidence of any participation of the cell nucleus in the formation of virus, inclusion body, etc. By contrast, this may occur in our virus, where the nuclei are situated directly on the side of the inclusion bodies and differ in internal organization from nuclei in uninfected cells. The development of the disease in the tissues causes increased division of fat body cells. This does not occur in Vagoiavirus infection.

Inclusion bodies. The protein inclusions in Vagoiavirus have a rather complicated way of maturation: from thin needle-shaped particles to large, oval, very resistant inclusions, a final form of maturation and the only stage where virus particles can be shown. In this group of viruses only a part of the protein crystals will mature to the final stage whereas the other part remain as spindles without structure (in *Melolontha*) or as long filaments (in *Operophtera*) (Weiser, 1969). As shown by Bergoin *et al.* (1968*b*), virus membranes and particles are formed in the cytoplasm of infected cells and occluded later in the inclusion bodies. But this does not explain the origin and function of the "empty" crystals. In our virus all inclusion bodies from the early stage of formation have virus particles in the interior and there are no "empty" protein masses present at all. The amount of virus particles in the inclusion bodies is much higher than in typical Vagoiaviruses. In infected cells from 1 to 6 inclusion bodies are formed in direct connection

with the nucleus. It is a fact that in cells with more inclusions the size of the inclusions is reduced as compared with the size of a single inclusion where solitary. The source of protein for their formation seems to be limited.

Virus particles. In our own material (Weiser, 1965, 1969) and in the original material in *Melolontha* grubs (Bergoin *et al.*, 1968*a*) the virus particles of typical Vagoiaviruses are oval to spherical with corrugated surface, similar to walnut shells. In the inclusion bodies, the same as when free in the cytoplasm, the particles have no distinct thick surface layer and in their interior there is a distinct central body with a crescent-shaped structure. In the material from *Camptochironomus tentans*, the virus particles are cushion-shaped, with two flat plates in the central part sandwiched together. In some publications, the Vagoiavirus particles have been compared with particles of the smallpox virus and the whole group has been brought into connection with poxviruses. Compared with morphological studies on smallpox virus, this virus of vertebrates is much more similar to the *Camptochironomus* — virus than to the Vagoiaviruses. In smallpox, the particle is cushion-shaped, with a central core of two sandwiched layers under a distinct covering membrane. This is what we find in the material described here. Identical in morphology are also the young empty membranes of oval shape, appearing in both infections. In Vagoiavirus, the mature particles are all subspherical, never cushion-shaped. A similar shape, but with smooth surface, can be demonstrated in some particles leaving the protein mass in the midge inclusions.

For the close similarity of the virus in *Camptochironomus* with that of smallpox we would like to propose to include the *Camptochironomus* virus into the poxvirus group until comparative studies will be performed and the taxonomic position of the pathogen fixed. The definition of the virus may be as follows:

Pox-like virus of *Camptochironomus tentans* causes infection of the fat body of midges, leading to mortality of infected larvae. The virus produces oval inclusion bodies 2—16 by 8—10 μ in the cytoplasm of infected cells, mostly adjacent to the nucleus. Virus particles, several hundreds in every protein crystal, are cushion-shaped, 200—250 by 270—300 by 130—150 nm, with rounded corners and a double platelet of the central core. All protein inclusions are filled with virus particles.

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Explanation of Micrographs:

Fig. 1. Section of the fat body of *Camptochironomus tentans* with inclusion bodies of the pox-like virus (p). Most nuclei are uninfected (n), and some are dark, located in infected cells (i), adjacent to inclusions. Mallory stain, ×2000.

Fig. 2. A — Inclusion bodies of the pox-like virus in the fat body of *C. tentans* (I) with adjacent nuclei (N) and groups of developmental membranes and young virus particles (D).
B — Developmental membranes of the pox-like virus in groups probably forming later the inclusion bodies.

Fig. 3. C — Virus particles in the interior of the inclusion body of *C. tentans* in all section-planes of the cushion-like bodies.
D — Free particles of the *Camptochironomus* pox-like virus with a distinct outer layer and a central core.
Length of scale: 2A — 5 μ; 2B — 1 μ; 3C, D — 0.1 μ.