CHLAMYDOZOA
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JAUNDICE OF SILKWORMS

TRANSLATION NO.

742

FEBRUARY 1963

U.S. ARMY BIOLOGICAL LABORATORIES
FORT DETRICK, FREDERICK, MARYLAND

CCBL: FD2-3742 (T-42-1)
JPRS: R-2990-D

26 February 1963

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Translated for:

U. S. CHEMICAL CORPS BIOLOGICAL LABORATORIES
Ft. Detrick, Md.

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U. S. DEPARTMENT OF COMMERCE
OFFICE OF TECHNICAL SERVICES
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Ohio Drive and Independence Ave., S.W.
Washington 25, D. C.

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JAUNDICE OF SILKWORMS

by

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Archives fur Protistenkunde,
1907, Vol 10, pp 358-364.

During my stay in Rovigno in 1903/4 I was occupied with the peculiar disease of silkworms, called "jaundice" by the breeders. I obtained the material from Mr. J. Bolle, Director of the [Austro-Hungarian] Agricultural Chemical Station in Görz, who had earned particular merits in connection with the investigation of this disease. For external reasons, however, the studies had to be interrupted and I have only now been able to bring them, in Hamburg, to a partial conclusion.

The jaundice attacks the silkworms in the breeding establishments especially at the time when they become ripe for spinning. The sick worms can be recognized at first by their lack of appetite; they crawl around unsteadily in the racks; their skin at first looks opaque, then becomes conspicuously shiny as a result of an inner tension; hence, these worms are also called "shiny worms (luisettes)." Later the abdomen of the worm is swollen, the skin is extremely easily injurable and there appear on it yellow spots which are confluent with one another and which actually give the disease its name, jaundice. Since the abdomen is conspicuously swollen, the disease is also called "fatty degeneration (grasserie)." From the tears in the skin which occur at every opportunity a milky yellowish liquid -the blood of the worm- trickles out. The Italian breeders call these worms vacca (cow). Finally the worms become limp, die, and flow apart into a brown sticky mass. A great many researchers had already been occupied with the jaundice of silkworms; of these I will only mention the names of E. Cornalia, Maestri, A. Cecconi, E. Verson, F. Haberlandt, Forbes, Panebianco and Bolle. A summary presentation of the research results was published in Padua, in 1896, by E. Verson and E. Quajat, entitled "Il Filugello e l'arte serica (The silkworm and the Art of Silk)."

In the blood of jaundiced spinners Maestri (Frammenti anatom. fisiolog. e patolog. del baco da seta [Anatomical, Physiological and Pathological Details of the Silkworm], Pavia, 1856) found a large number of granules which he related to the fat tissue; later E. Verson identified its crystalline nature. In 1893 Bolle (Jahrbuch der k.k. Seidenbau-Versuchstation [Yearbook of the Austro-Hungarian Silk Experimental Station], 1893, page 112) established that the so-called polyhedral particles consist of a protein substance.

These polyhedral particles are specific for the jaundice of silkworms.

The polyhedral particles are about 5 μ [in diameter], though their size not infrequently varies between rather wide limits. With sufficient magnification it can be established that they have a hexagonal contour and form rhombic dodecahedrons; in addition from time to time there may be seen octahedrons with truncated corners. They have a fairly high refractivity and possess a fatty shine. With pressure they acquire charateristic tears and may be cut up into irregular star- or cross-shaped rosettes. The peripheral sectors adhere together in a centrally multiple form. They are heavier than water and soon sink to the bottom of the test tube. According to Bolle who had investigated them very accurately, they are insoluble in boiling water, CS_2, alcohol, ether, chloroform, glycerol and petroleum ether, in the warm or cold state. They are further insoluble in hydrogen peroxide, benzene, saponine and sapotoxin. In ammonia solutions they first swell up, then from the periphery crystalline plates detach themselves from a substance which at first was crystalline but soon becomes grainy; these plates disappear gradually; finally there remains a transparent crystalline shadow which later also vanishes. - In glacial acetic acid the polyhedral particle will first swell up. The crystalline substance is permeated by radial rays analogously to a spherical crystal. These tender crystalline pyramids -which the crystal rays actually are- call forth, looked at from the surface, a tender honeycomb structure of the polyhedral particle (magnification 2,250), in each of which three pyramid bases meet. Already from these observations it is amply evident that the polyhedral particles possess an unusually complicated structure and that they consist apparently of two substance modifications, - a less refractive, presumably organic ground substance, and a strongly refractive crystalline mass which "permeates" the ground substance in the manner of a spherical crystal. Sulfuric acid makes the polyhedral body swell up, and one can observe at first a membrane-like "enveloping" of the crystals: the individual surfaces may be folded or centripetally dented by applying pressure with the cover glass, or by displacing the cover glass. Finally these last remains disappear and the particle is dissolved by sulfuric acid, nitric acid and acetic acid as well as by alkali. In general, depending on the state of their "maturity," the particles behave somewhat differently toward the above-mentioned reagents: some are dissolved quickly, while with others one may note an initial concentric stratification followed by a rather belated disappearance.

With 1% NaOH they are inflated to up to four times their initial size; however, they then melt together analogously to the gas vacuoles of the Arcella. On the basis of their ground substance they seem to belong to the protein family, and I assume that they are crystalloids of nucleoproteins. Their protein nature is borne out by the following: KI stains them yellow; on heating the color turns brown. In a freshly prepared state they give a positive Millon reaction (also noted by Bolle) which is obtained particularly readily with young particles which are still spherical. Analogously to other proteins they are stained yellow by picric acid. On addition of pepsin-HCl (32°C) they first become refractive, then fall apart into individual crumbling particles and finally disappear. With Giemsa's Eosin Azure a blue coloration is obtained especially in the younger forms of the polyhedral bodies; the larger crystalloids are tainted mostly on the periphery; Bolle stained them with aniline dyes such as fuchsine, eosin, erythrosine, Methyl Green, Meteor Blue, Gentian Blue and Methyl Violet. With Brilliant Cresyl Blue they take up a bluish tinge.

It is possible at all times to infect worms with the abdominal-cavity fluid of a worm which contains polyhedral particles; either one strokes fresh mulberry leaves with this material by means of a brush, lets dry and feeds the leaves to the worms, or one dips an ignited needle into the above-mentioned substances and then punctures the so-called false foot of a worm. Five to seven days after the infection the worms die of jaundice. As Bolle has shown, the virus possesses a rather high tenacity: he was able to infect worms and pupas via subcutaneous injection of "one-year-old blood, dried on glass plates," always obtaining positive results. The polyhedral particles are, however, not the carriers of the virus, since one is able to eliminate them by means of repeated filtration through several thicknesses of filter paper; then for control the filtrate is centrifuged and sedimented for an extended period of time; the clear, particle-free supernatant liquid which is subjected to an accurate microscopical examination in search for any of the articles in question that may possibly be present, may then be used to produce infection with a positive result.

Silkworms which had been infected in this manner at noon on 20 June 1904 died of jaundice on 24 and 25 June, and in their abdominal cavity a large number of typical polyhedral particles was detectable.

I conceive of the polyhedral bodies as specific reaction products of the host cells to the virus. I studied their genesis on smear preparations obtained from the blood of the abdominal-cavity fluid, and above all from the fat of the sick silkworms; these [smears] were prepared by dipping them while still wet, horizontally into a hot alcoholic sublimate solution.

Fixation: Two-thirds sublimate (concentrated hot saturated aqueous solution) + one-third 90% alcohol, 10 minutes.

Washing with distilled water; 60% iodine-alcohol [sic]; washing with 60% alcohol, then with distilled water.

Staining with dilute (commercial) Grenacher's hematoxylin, one quarter hour.

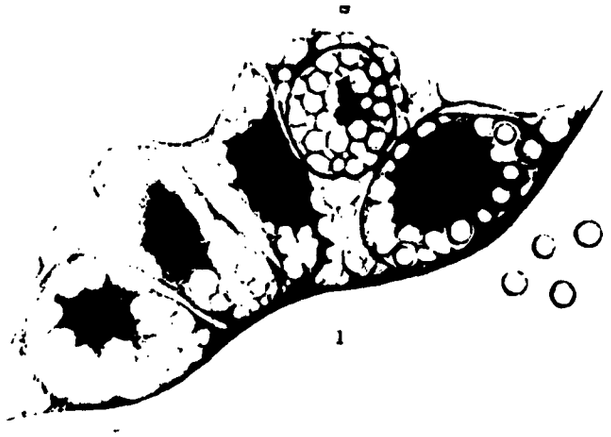
Washing with well water. Alcohol series [sic], xylene, Canada balsam.

In the diseased cells one first notes that the nucleus has become hypertrophic; the nucleolus is very much enlarged, is stained very dark in the blood cells; is often flapped, irregularly shaped in the fatty-body cells and possesses a minute honeycomb structure. The chromatin of the nucleus collects into individual lumps which adhere especially to the inner line of contour of the cell membrane (Fig. 1a). The first polyhedral particles emerge in the honeycombs of the achromatic framework; the number of these particles quickly increases. Later they enter also the protoplasm (Fig.1). Sometimes one finds in the smear also naked, very strongly hypertrophic oval nuclei of the fatty-body cells which are completely filled with polyhedral particles, while the chromatin adheres only to the nucleus membrane and so imulates a type of cyst membrane. At times the chromatin of the nucleus adheres to the free polyhedral particle in a hood-like manner. The polyhedral particles originate in this way at first intranuclearly on the nuclei of all tissues of the diseased worms; later they are detectable also in the protoplasm: when the cells fall apart the free particles flood the abdominal-cavity fluid and blood of the diseased worms.

At the edge of these smear preparations from the abdominal-cavity fluid and blood of jaundiced silkworms, where the serum had been fixated in a somewhat thinner layer, it was possible to detect very numerous light formations in the serum clot as soon as the preparations were stained intensively several times with Giemsa's Eosine Azure, and further worked up as dry cover-glass smears (that is, dried with blotting paper and enclosed in cedar oil). (Fig.2). In the light, oval to round small formations a reddish-violet or dark blue dot-like body, having the appearance of a coccus, could mostly be detected (magnification 2,250, using Auerlicht homog. immersion). These bodies at times separate into dumbbell shapes and I consider these formations as the actual causative agents of the jaundice of silkworms.

They are roundish, proliferate through a dumbbell-shaped cross division and appear to possess a gelatinous envelope (light border). I assign them the preliminary name of Chlamydozoon bombycis. They could be represented even better if one diluted the material strongly with distilled water, centrifuged intensively, washed several times and then stained the residue with Loeffler's flagella mordant. Unfortunately I do not have any material left for these experiments, and I plan to resume these investigations at a favorable opportunity. In individual cases I noticed [these bodies] also in the protoplasm of blood cells- these would be the intracellular stages of the chlamydozoon, to be compared

with analogous stages of the epithelial cells of vaccine-inoculated rabbit cornea.



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Figs. 1 and 2. Jaundice of Silkworms. Fig.1. Fat-Body Cells in Various Stages of Degeneration. At a nucleus is swollen, chromatin poor and completely filled with Bolle's polyhedral bodies, a few of which are drawn below in the free state.

Fig.2. Causative Agent of Jaundice (Oc. 8, hom. immersion 2 mm), drawn by Mr. Stender.

As a prophylactic measure against the jaundice of silkworms Bolle recommends immediate removal and burning of the first jaundiced worms, a more frequent change of the bedding of the latter and burning of the contaminated beds.

Bolle succeeded in positively infecting, by means of the virus of jaundice, also other insects such as Antherea Jama Mai and A. Pernyi, Attacus Cynthria, Antherea mylitta and larvae and beetles of Dermestes lardarius, only, interestingly, the polyhedral particles varied in shape from host to host. Since according to Bolle (1889) the jaundice occurs also on the feared Psilura monacha L., infection experiments on a large scale would be of particular importance, also from the national-economic point of view, especially since the virus, dried on glass plates, can be preserved over a year in a state capable of causing infection, and readily shipped in this manner.

I wish to express my sincere thanks to Director J. Bolle, Gorz, for his manifold advice and for the furnished material.

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