DEVELOPMENT OF NUCLEAR POLYHEDROSIS VIRUS IN SILKWORMS. *BOMBYX MORI* LARVAE AND ITS IMPACT ON SERICULTURE IN PUNJAB, N.W.F.P. AND AZAD KASHMIR.

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Summary. *Diseased larvae of silkworms were collected from different rearing areas of Pakistan and Azad Kashmir in* 1975-76. *Nuclear polyhedrosis virus was identified as one of the causal agents responsible for the death of millions of silkworm larvae in Pakistan and Azad Kashmir. Histopathological studies showed that the nuclei of adipose tissues, tracheal membrane, dermal cells and blood cells developed polyhedral bodies within a week after infection. Nuclear polyhedrosis virus multiplied rapidly and finally occupied the whole cell. Due to rapid increase in number of Nuclear polyhedrosis virus, the cells and finally all the tissues disintegrated, resulting in the death of the individual. Nuclear polyhedrosis virus infection in silkworm caused heavy losses to poor sericulturists.*

Introduction. Sericulture is a cottage industry in Pakistan and Azad Kashmir. During the last thirty years diseases have developed practically in all the races of *B. mori* and have caused tremendous losses to silkworm rearers.

Studies of Watanabe (1966), on relative virulence of polyhedrosis virus and host resistance in the silkworms showed that there existed a significant relationship between *B. mori* inter-strain susceptibilities to nuclear and cytoplasmic polyhedrosis viruses. In a preliminary study Jafri (1968), reported that environmental factors played an important role in the development of *Nosema bombycis*, Nuclear polyhedrosis virus, and bacterial infections in various races of silkworm reared in various ecological zones of Pakistan.

So far little published data is available on the development of Nuclear polyhedrosis virus infection in various races of *B. mori*. The present study was started dealing with the cytopathology of local strains of *Bombyx mori* larvae suffering from Nuclear polyhedrosis virus infection.

Materials and Methods. Diseased second, third and fourth instar larvae, pupae and cocoons of various races of silkworms were collected from different rearing areas of Punjab, N.W.F.P. and Azad Kashmir. In order to isolate the pathogen, the blood of infected silkworm was centrifuged at 5,000 rpm for 10 minutes. Four layers could be distinguished. The bottom layer consisted of a whitish sediment made up of polyhedra. Next to the bottom, there was a cellular layer of yellow debris. Then there was a layer of serum which was distinctly opalescent. On the surface of all, there was a pellicle of oil, which could be

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easily lifted off with the forceps moistened in water. The bottom layer was separated. A few drops were taken from the bottom layer and were spread on the slide and examined under light microscope. Smears showed a large number of 4-5 sided polygonal bodies. These bodies were identified as Nuclear polyhedrosis virus.

For histopathological studies the diseased larvae collected from Azad Kashmir were fixed at the spot in Bouin's fixative and Carnoy's fixative in the last week of April and first week of May, 1975. Fixed material, when brought to Lahore in the second week of May, was found disintegrated and was not fit for histopathological studies. However, the larvae fixed in the rearing area in formalin were brought to Lahore and found to be fit for histological studies.

This experience showed that during the hot months, at the time of collection, the diseased larvae should be fixed in formalin. However, in laboratory, these larvae can be fixed in any suitable fixative.

Histopathology of the larvae was carried out by the method as described by Vago and Amarger (1963). Serial sections of the larvae, 4-6 microns thick, were cut. Slides were stained by Jenner—Giemsa method as described by Humason (1962).

Results and Discussion. While conducting the survey, it was seen that in the diseased larvae appetite was decreased and their skin tension was lost. Usually five to seven days after infection, the intersegmental membranes of the body became swollen and the skin became shiny. At later stages, the larvae showed swellings at intersegments. The larvae became restless and impatient. The colour changed to light yellow and normally clear haemolymph became turbid. The integoment became fragile and when ruptured, a milky haemolymph flowed out. In the haemolymph of infected larva a large number of polyhedral bodies were observed. As the disease advanced, the larvae were excited, crawled aimlessly and fell off the rearing beds on to the floor where they crawled in a circle and died. The period from the swelling of the inter-segmental membranes to death was relatively short, from several hours to less than a day. If the disease occurred just before moulting, the period when it was most likely to occur in the early stages, the larvae did not moult earlier. A large number of larvae did not moult at all.

During the development of disease, no external change was observed in the pupae, but towards the end, the skin was easily ruptured on handling, as the pupal body was homogenized by that time. Usually black markings appeared on the body at the time of death. Most of the larvae died before pupal formation. Surviving larvae, span flimsy cocoons.

The haemolymph and faeces of the second, third and fourth instar larvae were examined in the post infection period. The blood cells showed a concentration of Nuclear polyhedrosis virus in the nuclei on eighth day of infection. Nuclear polyhedrosis virus was also seen in the faeces of the larvae on eight day after infection.

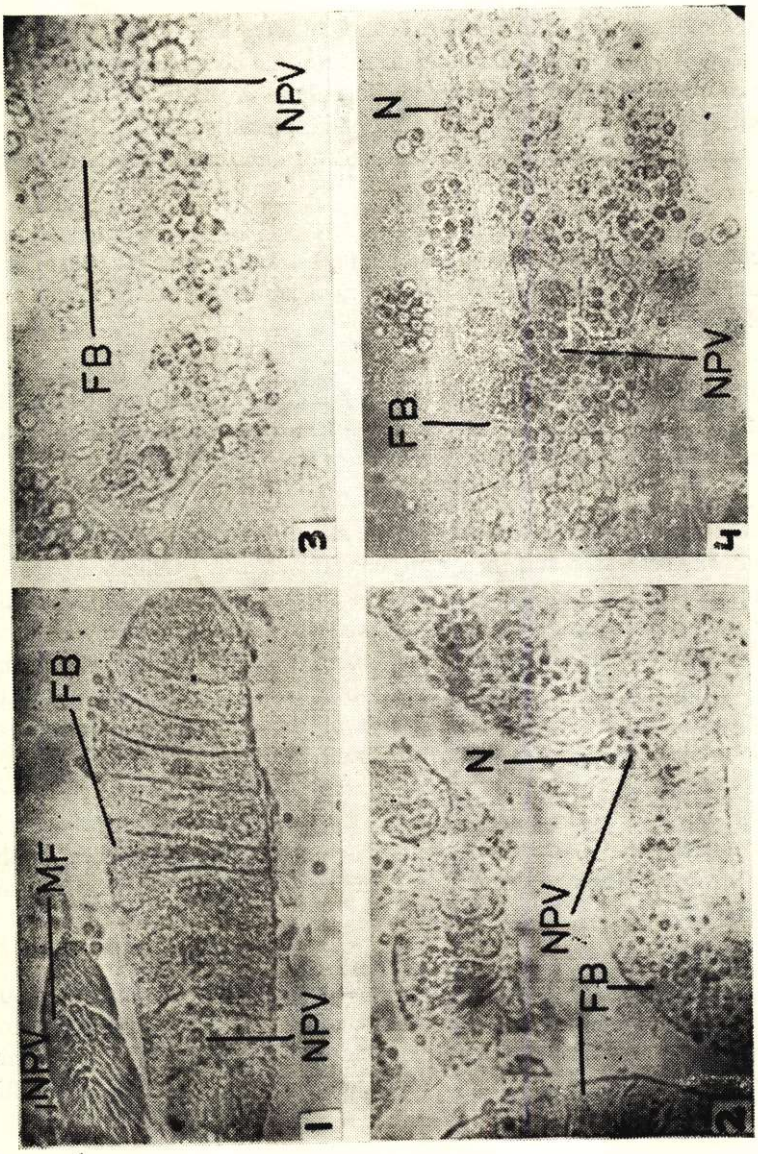

PLATE I

Fig. 1. Appearance of Nuclear polyhedrosis virus in fat body, (FB) X 400.
Fig. 2. Increase in size of fat body (FB) nuclei due to Nuclear polyhedrosis virus (NPV), X 1,000
Fig. 3. Disintegration of fat body (FB) tissue due to increase in number of Nuclear polyhedrosis virus (NPV), X 400.
Fig. 4. Various degrees of development of Nuclear polyhedrosis virus (NPV) in fat body (FB) nuclei X 400.

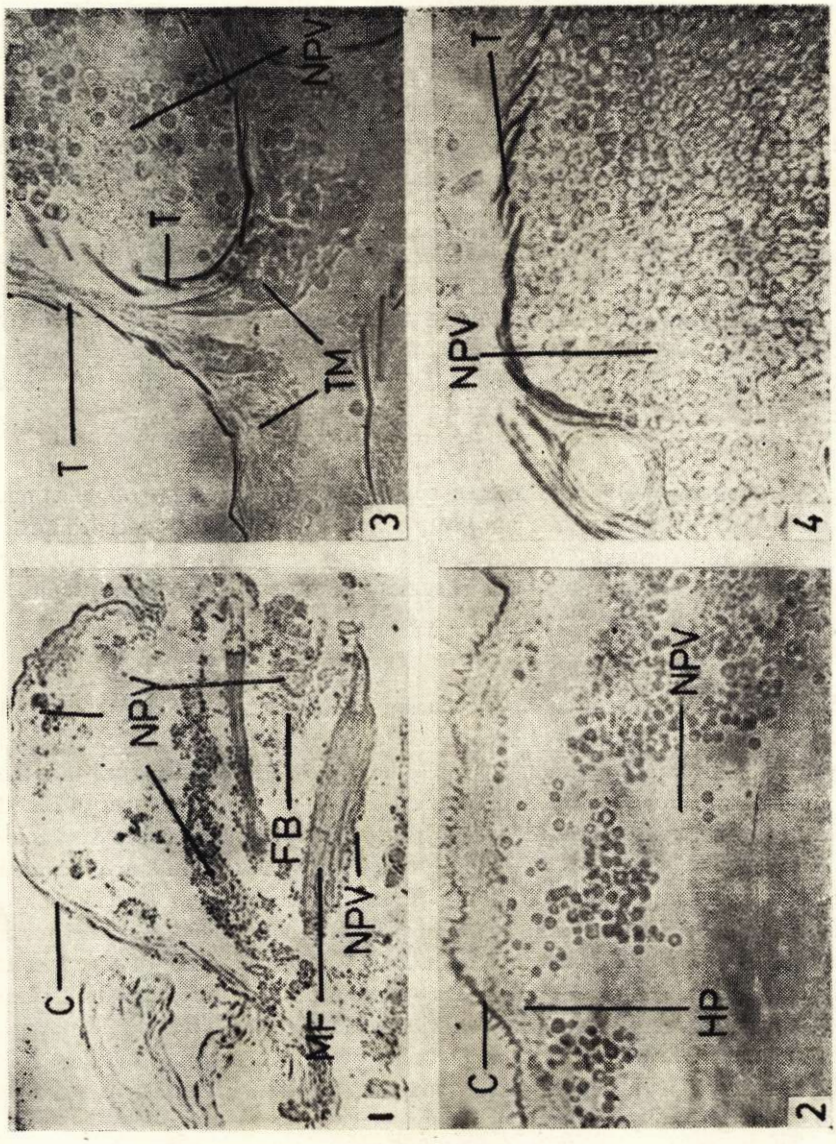

PLATE II

Fig. 1. Nuclear polyhedrosis virus (NPV) infecting muscle fibre (MF) and fat body (FB), X 400.
Fig. 2. Disintegration of hypodermal cell (HP) due to multiplication of Nuclear polyhedrosis virus (NPV), X 1000.
Fig. 3. Nuclear polyhedrosis virus (NPV) infecting tracheal membrane (TM) and lumen of trachea (T), X 400.
Fig. 4. A portion of trachea (T) showing lumen of trachea filled with Nuclear polyhedrosis virus (NPV), X 1000.

Histopathological studies showed that at an early stage of infection in silkworm, chromatin aggregated and very small granules appeared in the nuclear ring zone of fat body cells (Plat I, Figs. 1, 2). These granules appeared to be early stages in the development of polyhedra which grew around a central mass. Nuclei of infected cells increased in size. The increase in size of infected cells was also observed by Smith and Xeros (1953c) and Yamafuji (1952b). Nuclei which were filled with polyhedra, occupied nearly all the parts of the cells and finally cells appeared in a destroyed state by the fully grown polyhedra (Plate I, Figs. 3 and 4). Our histopathological studies showed that the polyhedral bodies were formed in the nuclei of adipose tissues, tracheal membranes, hypodermal cells and blood cells (Plate I. Figs, 1-4, Plate II, Figs 1-4). The formation of polyhedra in the middle and posterior portions of the silk glands was also noticed.

Histopathology of a fourth instar diseased larvae showed that tracheal membrane was heavily attacked by polyhedral bodies (Plat II, Fig 3). The polyhedral bodies even filled the interior of trachea (Plate II, Fig. 4). As the disease advanced the muscles of body cavity were attacked by polyhedral bodies (Plate II, Fig. 1). In a heavily attacked larvae, dermal cells were not seen, only a concentration of Nuclear polyhedrosis virus was seen below the cuticle (Plate II, Fig. 2).

According to Steinhaus (1949), the disease begins with the digestion of infectious material (polyhedra or free virus) into the alimentry tract of the animal. According to Day et al. (1958), Vago and Croissant (1959), and Bird (1959), the polyhedra are dissolved by the alkaline gut juice, and the liberated virus particles penetrate through the gut epithelium and multiply in the cells of the blood and other tissues.

Descriptions of the pathological changes occuring in jaundiced silkworms have been presented by Glaser (1927) and by Paillot (1930b, 1933), and the following account is based upon their observations. It has been established that one of the earliest indications that the presence of disease may be identified by examining the blood. The principal types of blood cells or hemocytes in normal silkworms are: leucocytes (40 to 50% of blood cells), Proleucocytes (25 to 40%), lymphocytes (10 to 15%) (proleucocytes are commonly included with the lymphocytes), spherule cells (10-15%) and a very small number of oenocytoids. In virus disease silkworms, the leucocytes and lymphocytes are the blood cells in the nuclei of which one may observe the development of polyhedra. When the nuclei of these cells (polyhedra are never found within the nuclei of the spherule cells or the oenocytoids) are filled with the inclusions, it is certain that the larva will succumb to the disease. The origin of the inclusion in the nuclei of the blood cells is preceded by a concentration of the nuclear substance and the formation of a central denser mass around which refractive granules appear. These granules gradually develop into polyhedra and eventually completely fill the nucleus. The cells finally become disorganized and liberate the polyhedra, which float in the haemolymph. The milky appearance of the blood of heavily infested caterpillars is due to the presence of these polyhedra together with the fat droplets being liberated from the disintegrating fat body. Thus, by frequent examinations of the blood, it is possible to find out the presence and extent of polyhedrosis disease in silkworm larvae.

Aizawa (1970), while discussing defence reactions of *E. mori* against the Nuclear polyhedrosis virus, mentioned that defence reactions in the silkworms against the Nuclear polyhedrosis virus covered several phenomena including resistance to virus disease and chemical therapy. Our histological studies have enabled us to know the details of development of Nuclear polyhedrosis virus in various tissues of *B. mori* larvae. This study will further help us to investigate the phenomenon of resistance of host tissues to virus infection and the effect of chemical therapy which may be applied to various races of virus infected silkworms reared in various ecological zones of Pakistan and Azad Kashmir.

Our survey showed that Nuclear polyhedrosis virus developed in all the races of silkworms, reared in various ecological zones of Pakistan. The disease caused a heavy mortality of larvae. The surviving larvae span cocoons of very poor quality. Thus the poor rearers faced heavy losses.

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