Int. J. Indust. Entomol. Vol. 2, No. 2, 2001, pp. 149~153

International Journal of *Industrial Entomology*

NADP-Dependent Malate Dehydrogenase Activity and Associated Biometabolic Changes in Hemolymph and Fat Body Tissues of Silkworm *Bombyx mori* L. Following Baculovirus Infection

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(Received 14 March 2001; Accepted 2 May 2001)

The influence of baculovirus *Bombyx mori* Nuclear Polyhedrosis virus (BmNPV) infection on intermediary metabolic pathways in silkworm *Bombyx mori* L. was investigated. Studies revealed that NADP-linked malate dehydrogenase activity in hemolymph of infected silkworms at 96 hrs post infection (p.i.) with visible symptoms of infection was enhanced in comparison to healthy larvae of the same age. Also, NADP-dependent MDH activity was significantly lower in fat body cytosol of infected larvae at 96 hrs p.i. when compared to healthy larvae. Similarly, some biometabolic parameters like growth, protein content and cholesterol titer were observed to be influenced by baculovirus infection. While the growth of infected larvae was significantly retarded, protein content was also drastically reduced in both hemolymph and fat body tissues. Cholesterol titer, however, was enhanced in infected larvae. The results observed herein point to a significant change in the normal biochemical and biometabolic pathways required for growth and development following BmNPV infection.

Key words : *Bombyx mori* nuclear polyhedrosis virus (BmNPV), NADP-malate dehydrogenase, Cholesterol, Silkworm.

Introduction

The silkworm *Bombyx mori* L. is susceptible to a number of viral diseases of which nuclear polyhedrosis virus accounts for more than 60% mortality. Nucleopolyhedrovirus infection in silkworms caused by BmNPV (*Bombyx mori* nuclear polyhedrosis virus) is characterized by polyhedral shaped occlusion bodies (OBs) within the nuclei of susceptible cells. It is known that the larvae of *B.mori*, the silkworm, infected with BmNPV die within several days.

Several reports are available on the biochemical changes which occur during the course of nucleopolyhedrovirus infection. Watanabe *et al.* (1972) elucidated the changes in the synthesis of hemolymph proteins in *B.mori* on infection with a nuclear and cytoplasmic polyhedrosis viruses. Biochemical changes in hemolymph of the silkworm during progressive infection of nuclear polyhedrosis virus has been studied by Sharma *et al.* (1994). Similar studies (Watanabe and Kobayashi, 1969; Shigematsu and Noguchi, 1969; Kobayashi and Kawase, 1980; Mikhailov, 1992) indicated the profound biochemical changes which occur in the silkworms following viral infection. However, scant information is available on the influence of baculovirus infection on intermediary metabolic pathways coupled with changes in some important biomolecules.

The present study was undertaken with a view to elaborate on the changes in biometabolic pathway and some other biochemical parameters occuring as a result of BmNPV infection in silkworms and to speculate on their possible physiological significance.

Materials and Methods

Insects and tissue preparation

An evolved multivoltine race of *Bombyx mori* L (race G) was reared with fresh mulberry leaves at 25 ± 1°C and 12 L + 12 D photoperiodic condition. Three days old fifth

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instar larvae were selected for the experiment. 150 larvae were kept in three separate replications of 50 larvae each for treatment and control lots. Polyhedra of BmNPV was purified on Percoll cushions (PVP coated silica particles, Sigma Chemical Co. USA) and an inoculum (3.6×10^9 OBs/ml) was inoculated perorally (by smearing on 9.125 sq.cm. mulberry leaf disc) to silkworms at 0 hour (3rd day 5th instar).

Silkworms were sacrificed from treated and control groups at 0 hr and 96 hrs post inoculation for collection of hemolymph and fat body tissue. Hemolymph was collected from treated and control groups by amputating a proleg and collecting the exuded hemolymph in a 1.5 ml sterile eppendorf tube coated with phenyl thiourea for preventing melanization. Cells and debris were removed by centrifugation at 4°C for 10 min at 3,000 g in a Sorvall RC5C centrifuger.

The fat bodies from the silkworm larvae were surgically removed in cold and rinsed with cold 0.65% saline to make it free from hemolymph contamination. Specific amount of tissue was homogenised in 0.25 M sucrose in a glass- teflon homogenizer. Cell debris and nuclei were separated by centrifuging at 5,000 g for 5 min. The supernatant was centrifuged at 16,000 g to precipitate the mitochondria and the supernatant (post mitochondrial fraction) was considered as the cytosol.

Enzyme assay

The cytosolic and plasma NADP-linked malate dehydrogenase (EC 1.1.1.40) was assayed following the method of Hsu and Lardy (1969) as modified by Murphy and Walker (1974) with slight modifications for insect tissue by using a reaction mixture containing 0.5 mM NADP, 3.8 mM L-malic acid, 0.05 mM $MnCl_2$ and 48 mM triethanolamine buffer at pH 7.4. The rate of NADP reduction was measured at 340 nm in a Shimadzu (UV-160A) spectrophotometer and specific activity was expressed as Δ Abs / min / mg protein.

Protein and cholesterol assay

Protein content in hemolymph cell free plasma and in fat body tissue cytosol was determined by the method of Lowry *et al.* (1951) and absorbance was read at 750 nm in a Shimadzu spectrophotometer. Bovine serum albumin (Sigma, USA) was used as protein standard.

Cholesterol content in hemolymph plasma was assayed using the method of Kabara (1962) and readings were taken at 540 nm. Cholesterol (Sigma) was used as standard. Data presented are mean ± standard error of observations of five individuals of each replication in each group. Statistical analysis of data was made by Students 't'- test.

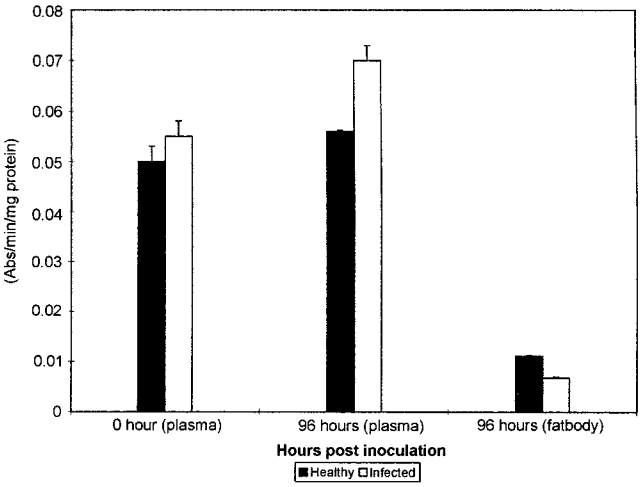

Fig. 1. BmNPV induced changes in NADP-dependent malate dehydrogenase activity in hemolymph plasma and fat body cytosol of female *B. mori*. The silkworms were sacrificed at 0 hr (3 day 5th instar) and 96 hrs (7day 5th instar) post inoculation. Each mean value is the average of 5 replications and vertical bars represent the standard error of means.

Results and Discussion

Changes in NADP-linked malate dehydrogenase activity in hemolymph and fat body cytosol

NADP-linked malate dehydrogenase (decarboxylating, EC 1.1.1.40) a key enzyme of the intermediate metabolic pathway plays an important role in the conversion of L-malate to pyruvate. The enzyme was found to be present in hemolymph and fat body tissue of silkworm. It was observed that the activity of this enzyme was substantially enhanced ($p < 0.001$) in hemolymph plasma in infected larvae when observed at 96 hrs post infection i.e. after the appearance of full blown symptoms of polyhedrosis disease (Fig. 1).

On the other hand NADP-MDH activity was significantly lower ($p < 0.001$) in fat body cytosol of infected larvae when compared to healthy larvae of same age. Both NAD- and NADP-linked malate enzymes have been found in insect tissues. The NADP-linked enzyme reported as present in the fat body of *Periplanata americana* is almost exclusively a cytoplasmic enzyme (Storey and Bailey, 1978b). Its function is probably in synthetic reactions such as the formation of fatty acids.

In the early stage of virus multiplication, the catalase activity in the hemolymph decreases, and after the release of polyhedra into the hemolymph the activity markedly increases (Yamafuji, 1959). It is also known that polyhedra contain a small amount of phospholipid (Wyatt, 1952; Bergold and Wellington, 1954; Ishimori, 1957; Faulkner, 1962). The lipid content in the hemolymph of BmNPV

infected silkworm is higher than that of normal larvae (Komano *et al.*, 1966). The lipid fraction consists principally of phospholipids which increase 2.5 fold in infected larvae in comparison to the normal silkworm. It seems that the specific role of phospholipids is to form polyhedral protein or virus protein directly or indirectly, as it has been reported by Hendler (1959) that phospholipids plays an important role in the biosynthesis of protein.

In conjunction to the above facts, it is quite plausible that enzymes involved / required for intermediary metabolism are intracellular and in health only small amounts are present in plasma, but when some disease process leads to increased cell breakdown in an organ enzymes may escape in greater quantity with consequent increase in their activity in plasma. This may be the reason for increased activity of NADP-MDH in serum as found in BmNPV infected silkworms. Moreover the decrease in activity of NADP-MDH in fat body cytosol of infected larvae points to their mobilization and degradation of fat body tissue normally associated with this disease. The extent of the rise in serum activity of NADP-linked malate dehydrogenase depends not only on the concentration of the enzyme in the tissue and the extent to which the organ is damaged but also on the rate at which the enzyme escapes from the damaged tissue, the location of the enzyme in the cells, changes in permeability of cell membranes and the rate at which the enzyme is removed from the serum either by metabolism or by renal excretion.

Changes in protein content

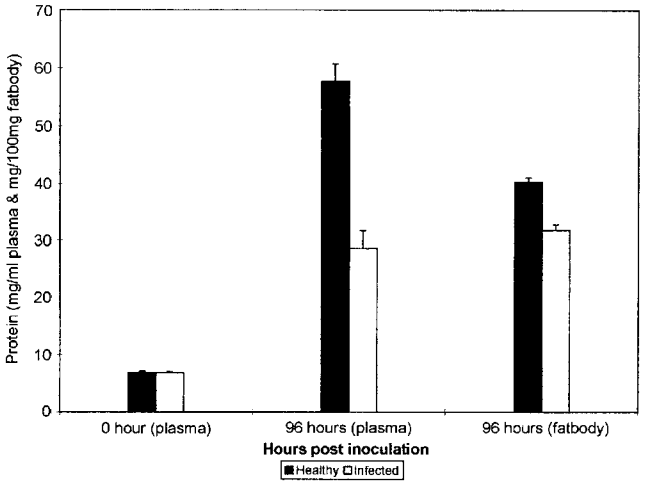

Fig. 2. BmNPV induced changes in protein content in hemolymph plasma and fat body cytosol of female *B. mori*. The silkworms were sacrificed at 0 hr (3 day 5th instar) and 96 hrs (7day 5th instar) post inoculation. Each mean value is the average of 5 replications and vertical bars represent the standard error of means.

The protein content in hemolymph and fat body tissue was found to be markedly reduced ($p < 0.001$) following the appearance of disease symptoms (96 hrs p.i.) in comparison to healthy larvae (Fig. 2). The hemolymph serum is the only extracellular fluid in insect and among its primary functions are the storage and translocation of metabolic products throughout the body. It is also well known that the physiological changes occur in the hemolymph during the course of a nuclear polyhedrosis virus infection (Aizawa, 1963; Benz, 1963; Bergold, 1964; Martignoni, 1964). These changes at least in part may be traced to the fat body which plays a dominant role in insect metabolism and is a major site of virus replication. Since, the fat body is the primary site of hemolymph protein synthesis (Wyatt and Pan, 1978). Protein content profiles should show changes reflecting protein synthesis and release in infected fat body.

Young and Lovell (1971) reported that hemolymph protein concentration in diseased larvae (following NPV infection) of *Trichoplusia ni* decreased in comparison to healthy larvae. This is in concurrence with our own findings in the evolved race 'G' of silkworm *B.mori*. The difference in protein concentration between healthy and diseased larvae becomes more pronounced as the disease progresses, indicating that after NPV infection of fat body cells normal protein synthesis and release is greatly reduced.

The large reduction in protein concentration in hemolymph at 96 hrs p.i. occurred at a time when most fat body cells were infected. This reduction late in the disease (i.e. appearance of external disease symptoms) may be due to sequestration of protein by infected cells for virus encoded protein synthesis, particularly that of occlusion body development for which large quantities would be needed. Information concerning this process in virus infected cells is lacking though it is known that hemolymph proteins are temporarily stored by healthy fat body cells (Locke and Collins, 1966).

Protein content in fat body was also noted to be significantly ($p < 0.001$) lowered during BmNPV infection in silkworms. It is known that most lipids are transported as conjugates in the form of diglyceride-protein complexes (Gilbert, 1967). Therefore, a loss of normal lipoproteins during this disease would be expected to result in a reduction of lipid transport throughout the body cavity. This is contrary to our observations (unpublished data) and to the findings of Komano *et al.* (1966). The release of lipids into the hemolymph may be due to the rupture of cytomembranes of polyhedra filled cells. This is in agreement with the findings of Young and Lovell (1971) who reported the presence of a new lipoprotein in virus infected cells. These are capable of transporting lipids from

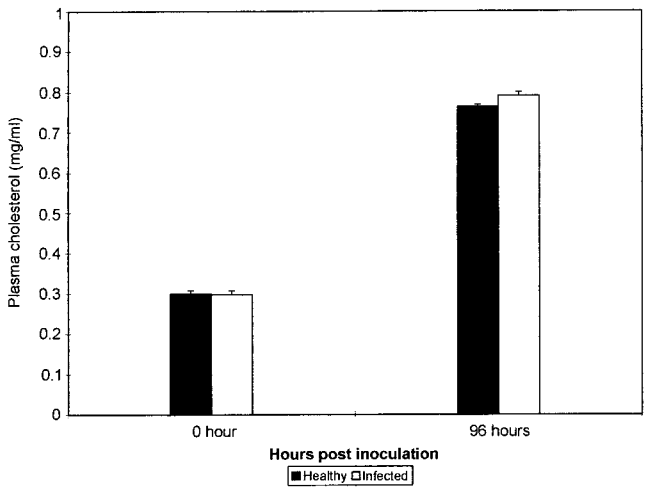

Fig. 3. BmNPV induced changes in plasma cholesterol of female *B. mori*. The silkworms were sacrificed at 0 hr (3 day 5th instar) and 96 hrs (7day 5th instar) post inoculation. Each mean value is the average of 5 replications and vertical bars represent the standard error of means.

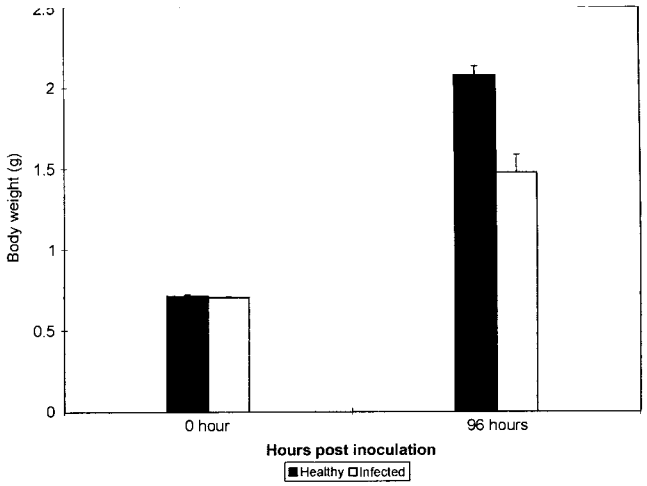

Fig. 4. Changes in larval weight during 5th instar after Bm NPV infection of female silkworm *B. mori*. The weights recorded were the average of 20 individuals, vertical bars represent the standard error of the means.

degraded fat body tissue to hemolymph serum and are suggested to be part of cytomembranes which have been liberated into the hemolymph after lysis of infected cells.

Changes in cholesterol content

The cholesterol content in infected larvae was greatly enhanced ($p < 0.05$) when assayed at 96 hrs p.i. when compared to healthy larvae (Fig. 3). The increase in cholesterol content in NPV infected silkworms, *B. mori* was also reported by Komano *et al.* (1966). Hemolymph in insects, as mentioned, is indispensable for different physiological processes. It has widely varying proportions of different inorganic and organic substances of physiological importance (Wyatt and Pan, 1978). Among the various nutrients present in the plasma of insects, protein and cholesterol are important components required for growth and development. The vital activities like growth, development, moulting, oogenesis, egg production and hatching in insects are influenced by cholesterol, a major phytosterol (Gilbert, 1964).

The metabolic role of cholesterol with respect to virus infection and formation has not yet been fully investigated. The changes in hemolymph composition, particularly protein and cholesterol contents in infected and healthy silkworms might be indicative of the changes in metabolic activities of some vital organs like fat body, gonad and endocrine organs. In the normal course of development, variations in cholesterol content are encountered during larval, pupal and adult stages corresponding to the increased / decreased formation of cholesterol from food sterols and / or from simple precursor as acetate in fat body and increased or decreased demands for tissue differentiation. The increased content of cholesterol in hemolymph of infected larvae may be a reflection of the decreased utilization of cholesterol by various tissues during development. This may have some far reaching effects in the larval-pupal and pupal-adult transformations as well as fecundity.

Change in body weight

The body weight of infected larvae in general was observed to be less ($p < 0.001$) than that of comparable healthy individuals (Fig. 4). The retardation in growth and development of infected individuals is borne out by the biometabolic changes enumerated above. On BmNPV infection the development of silkworms is markedly retarded, so much so that larval - pupal transformation was delayed by one full day (unpublished observation).

In conclusion it may be stated that in the silkworm *B. mori*, NPV infection produces profound changes in the activity of certain enzymes involved in intermediate metabolic pathways as well as on the concentration of various biomolecules. Since both hemolymph and fat body tissues play a dominant role in general metabolism, an investigation of these tissues during the course of the disease should yield some important information on the role of various metabolites as well as the nature of changes occurring in them during disease conditions.

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polyhedrosis virus; in *Insect Pathology an Advanced Treatise*. Steinhaus, E. A. (ed.), pp. 381412, Academic Press, New York.

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