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EFFECT OF BACULOVIRUS INFECTION ON CARBOHYDRATE, PROTEIN AND AMINO ACID LEVELS, AND PROTEASE ACTIVITY IN HAEMOLYMPH OF *ORYCTES RHINOCEROS* GRUBS*

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ABSTRACT

The effect of baculovirus infection on the levels of sugars, proteins and aminoacids was studied in the haemolymph of third instar grubs of *Oryctes rhinoceros*. Baculovirus infected grubs maintained almost identical levels of total sugars as the healthy grubs at different stages of analysis. Total protein level was higher in the healthy grubs when compared to that in diseased ones, but reverse trend was observed in protease activity and amino acid levels. The d14 post-infection was the critical phase at which drastic variations of the macromolecules occurred in the haemolymph.

INTRODUCTION

Baculovirus infection in the grubs of the coconut rhinoceros beetle, *Oryctes rhinoceros* was first reported by Huger (1966) from Malaysia. Since then, the virus has been used as an efficient entomopathogen for the biological suppression of rhinoceros beetle population in different coconut growing tracts of the South Pacific Islands, Fiji, Mauritius, Seychelles, Papua New Guinea (Bedford, 1981; Caltagirone, 1981) and in the Lakshadweep islands of India (Mohan *et al.* 1989). Intensive studies on the virus and its pathogenesis in grubs and beetles have been carried out earlier (Bedford, 1981).

However, the pathophysiology of the viral disease has not been fully understood. The effect of virus infection on the haemocytes of grubs was studied by Martin Jude Vincent *et al.* (1988). Baculovirus of *Oryctes* has been recognized by the FAO as one of the important entomoviruses for commercial exploitation. It is documented as one of the land-mark examples of successful biological control using an introduced entomopathogen (Caltagirone, 1981). Hence, it is imperative to know the effect of virus infection on the metabolism of the insect for better utilization of the pathogen. It is with this objective in view, studies on the effect of the virus infection on protein, amino acid and sugar levels, and protease activity in the haemolymph of the host were undertaken.

MATERIALS AND METHODS

Experimental insects

Eggs and the various instars of *Oryctes rhinoceros* grubs were collected from rotting coconut stumps and logs, and reared in sterile cattle dung contained in plastic boxes. The field collected grubs were kept under observation for atleast one week to check for natural infection. The entire lot was discarded even if one of the grubs developed disease symptoms. Eggs, either collected from the field or laid by laboratory reared beetles, were reared aseptically. Third instar grubs were used for the experiments because these are more sensitive (LD_{50} 1.3 x 10^{-3}) to baculovirus infection than first and second instars (Mohan *et al.* 1985).

(a) *Infection of grubs*

Midgut (1g) of virus infected, moribund

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grubs was macerated with 10 ml phosphate buffer (0.05 M, pH 8.5) to obtain 10^{-1} gut extract equivalent of virus inoculum. Log dilutions of the extract with the same buffer was made upto 10^{-3} level of dilution and used to infect the grubs. Third instar grubs were forcefed with 0.2 ml of the inoculum and maintained in sterile cattle dung. Control grubs were forcefed with 0.2 ml phosphate buffer.

Two grubs each from the diseased (d 4 post-infection (PI) onwards) and healthy lots were taken out and HL (haemolymph) was collected in a watch glass through one of the amputated prolegs and allowed to clot. The clot was removed and centrifuged (5000 g, 10 min, 4°C). The supernatant was used for analysis.

(b) *Estimation of protein content*

Protein content in the HL was determined as per the method of Lowry *et al.* (1951) as bovine serum albumin equivalents.

(c) *Amino acids*

The amino acid content of the HL was estimated following the method of Moore and Stein (1948) using leucine as standard.

(d) *Total sugar*

Total sugar level of HL was determined according to Gilbert (1957) as glucose equivalents.

(e) *Protease activity*

Protease activity of HL was estimated according to the procedure described by Laskowski (1955). Casein (20 mg/ml) was incubated with the HL for 20 min at 37°C. Enzyme activity was determined by evaluating the change in absorbancy at 280 nm in a Beckman UV-VIS spectrophotometer. Enzyme protein was estimated as per the method of Lowry *et al.* (1951).

RESULTS

Changes in total sugar levels in the HL of healthy and baculovirus infected grubs are depicted in Fig. 1. The trends in both healthy and diseased grubs were almost identical.

Changes in protein levels in the HL of the grubs are furnished in Fig. 2. The profiles of the total protein content of healthy and diseased grubs changed oppositely. In healthy grubs protein level started to decline from d-4 upto d-9 and raised again upto d-14 to the level of d-4, followed by a second decreasing trend. Conversely, the protein level of the diseased grubs almost doubled on d-9 post-infection and then declined upto d-14 post-infection followed by an increasing trend. Generally, the total protein content of the HL of healthy grubs was higher than that of the diseased grubs.

Effect of baculovirus infection on the total free amino acids in HL is furnished in Fig. 3. The amino acids (AA) profiles of healthy and virus infected grubs were almost identical. Though non-linear, the amino acid levels increased gradually upto d-14 and then declined sharply.

The protease activities of the HL of infected and healthy grubs are presented in Fig. 4. The activity profiles in both healthy and diseased grubs were almost identical, but the activity was significantly higher in diseased grubs from d-13, post-infection. Peak levels of protease activity were recorded on d-18 and d-15 in diseased and healthy grubs, respectively.

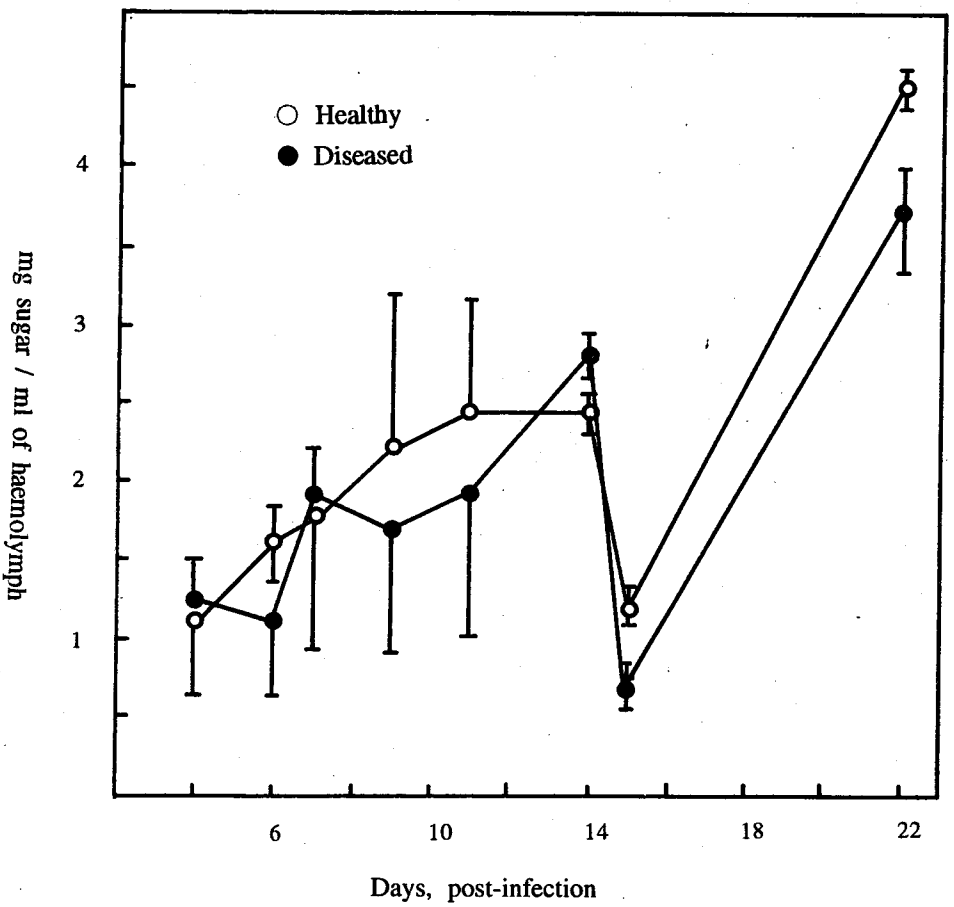

Fig. 1. Changes in total sugar levels (glucose equivalents) in the haemolymph of healthy and baculovirus-infected *Oryctes rhinoceros* grubs. Results are the mean of five replications.

DISCUSSION

Identical changes in the levels of carbohydrates in diseased and healthy grubs of *O. rhinoceros* (Fig. 1) suggest that baculovirus infection has insignificant effect on carbohydrate profile and supported the observation of Gujar and Chaudhari (1983), though Mohan *et al.* (1985) reported starvation symptoms in diseased *O. rhinoceros* grubs from d-5 PI. *Oryctes* baculovirus initially and primarily replicates in the midgut and afterwards in the fat cells and other tissues (Huger, 1966; Payne, 1974), so, decline of absorption of metabolites should reduce carbohydrate level in HL concomitant to NPV, GV, CPV, TIV and DNV infections (Morris, 1962; Martignoni, 1964; Van der Geest and Craig, 1967; Pawar and Ramakrishnan, 1975). Probably diseased grubs might have used stored food i.e. glycogen and maintained carbohydrate level in HL (Fig. 1) like virus infected or starved insects which utilize preserved food effecting loss of glycogen and simultaneous decline of hexokinase activity in HL (Morris, 1962; Martignoni, 1964; Van der Geest and Craig, 1967; Wyatt, 1967; Kaplan and Ben-Porate, 1968; Wigglesworth, 1972).

Trends of change of carbohydrate level in healthy grubs (Fig. 1), which probably reached

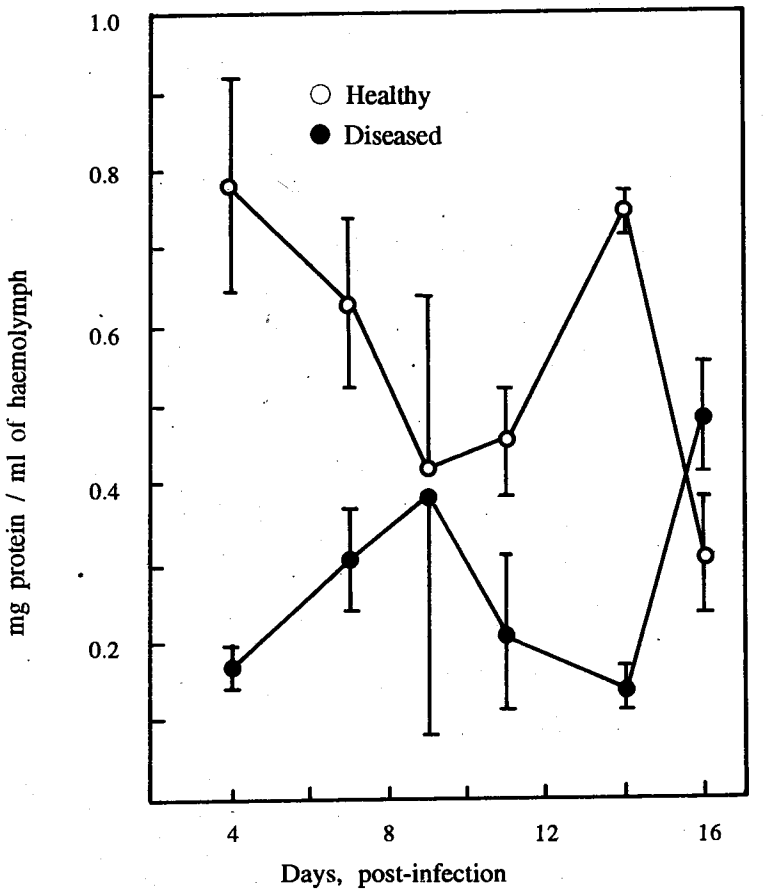

Fig. 2. Changes in total protein levels in the haemolymph of healthy and baculovirus infected *Oryctes rhinoceros* grubs. Results are the mean of five replications.

prepupal stage on about d-14 to 16 PI, conforms with normal carbohydrate metabolism which varies with periodic metamorphotic changes but level increases upto last instars (Wyatt, 1967; Wigglesworth, 1972).

The lower levels of protein in diseased grubs (except on d-16 PI (Fig. 2), as well as, about four-fold increase of protease activity on d-16 PI (Fig. 4) revealed pronounced effect of baculovirus infection on protein metabolism. The level of amino acids (AA) did not show much variations except slight reduction on d-4 and increase on d-14 PI in infected grubs compared to that in healthy ones (Fig. 3). During occluded (NPV, GV, CPV) and non-occluded (DNV, TIV) viral infections, it was observed that protein and AA level increase upto h30 PI, then decline below those of healthy insects upto d-4 PI, subsequently increase upto d-4 to 8 PI and then come down again upto d-7 to 10 PI (Martignoni, 1964; Martignoni and Milstead, 1964; Van der Geest and Craig, 1967; Shigematsu and Noguchi, 1969; Young and Scott, 1970; Morris, 1971; Shapiro and Ignoffo, 1971). Similarly, in bacterial, fungal and protozoan infections, and parasitization also hypoproteinemia was observed on d-4 PI (Bennet

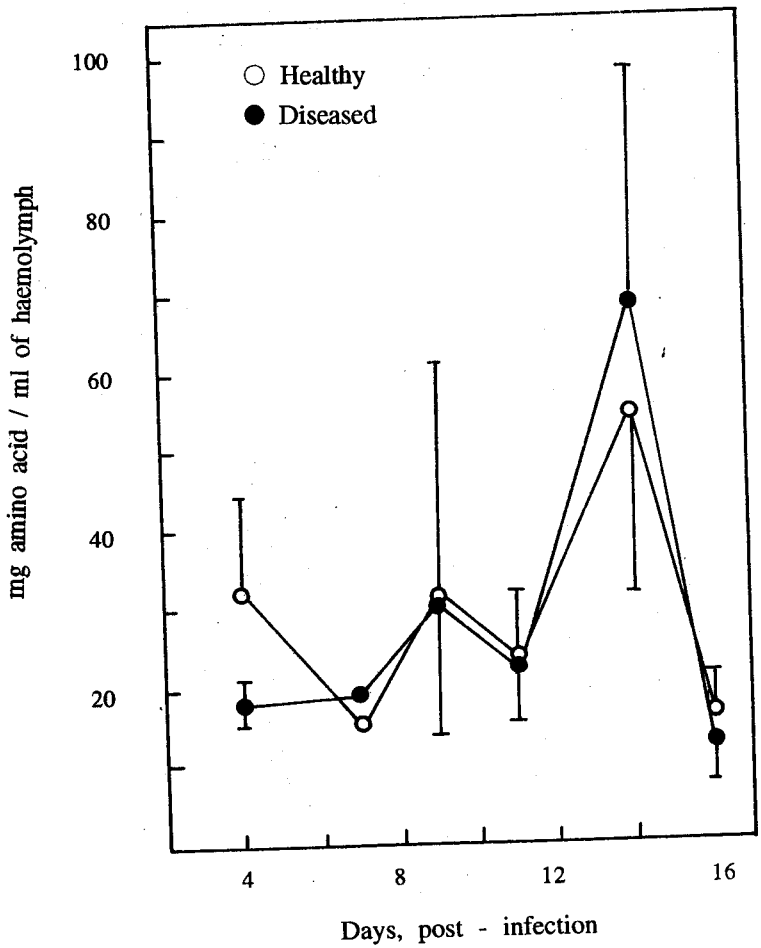

Fig. 3. Changes in total amino acid levels in the haemolymph of healthy and baculovirus infected *O. rhinoceros* grubs. Results are the mean of five replications.

et al. 1968; Newton *et al.* 1983; Cheng and Xiang, 1984; Rabindra *et. al.* 1987). Consistently lower level of protein (except on d-16 PI) in diseased grubs than that of healthy ones (Fig. 2) also conform with earlier observations (Shigematsu and Noguchi, 1969). About six-fold depletion of protein content in the diseased grubs (Fig. 2) indicates major metabolic upset within d-4 PI. During *Oryctes* baculovirus (Kelly, 1976; Crawford and Sheehan, 1985), NPV, GV, CPV, TIV and DNV infections also major cellular events take place within h30 PI. Alterations in haemolymph occurs from d-3 to 4 PI and cell lysis starts from d-4 PI (Shigematsu and Noguchi, 1969; Young and Scott, 1970; Shapiro and Ignoffo, 1971; Kelly and Tinsley, 1974). Temporal observations revealed initial rise o protein and AA in infected cells with h-6 (CPV and h-25 (NPV,GV) and second enhancemen at h-27 to 28 for inclusion body (IB) formatio which are gradually released after about d- to 4 PI due to cell lysis and slowly raisin protein level simultaneously in HL (Shigemats and Noguchi, 1969; Young and Scott, 197

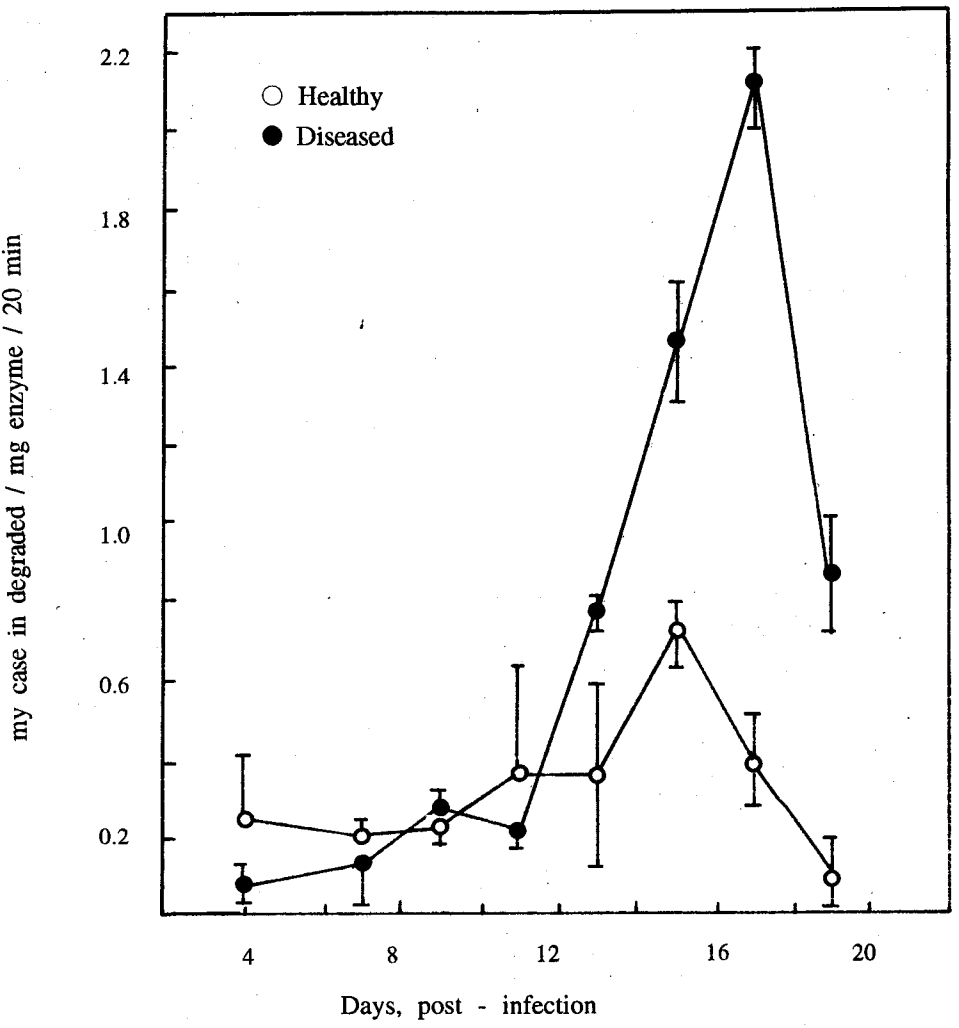

Fig. 4. Changes in protease activity in the haemolymph of healthy and baculovirus infected *O. rhinoceros* grubs. Results are the mean of five replications.

Shapiro and Ignoffo, 1971; Takei and Tamashiro, 1975). So, in baculovirus infected grubs, protein level decrease on d-4 PI (Fig. 2,3) due to continued inhibition of host protein synthesis and simultaneous degradation of HL protein. The decrease in AA level could be due to high rate of utilization of AA for virus specific protein synthesis. Similar alterations in protein and amino acid levels have been reported in NPV and GV infections which are identical (including replication) to *Oryctes* baculovirus (Kelly, 1976; Crawford and Sheehan, 1985) and CPV diseases of insects (Van Der Geest and Craig, 1967; Shigematsu and Noguchi 1969; Young and Scott, 1970; Shapiro and Ignoffo, 1971; Takei and Tamashiro, 1975). The gradual increase of HL protein from d-4 to 9 PI (Fig. 2) might be due to release of virions, as well as, contamination of cellular proteins due to gradual lysis of cells (Shigematsu and Noguchi, 1969; Young and Scott, 1970; Kelly, 1976). Lysis of cells would also release cellular proteases and increase its

level gradually in HL (Fig. 4) which in turn degrade more protein superseding AA requirement for virus replication and enhance AA level upto d-9 PI (Fig. 3). By d-12 to 14 PI, almost all cells might be on the verge of completion of cycles of viral replication (Shigematsu and Noguchi, 1969; Young and Scott, 1970; Payne, 1974; Kelly, 1976; Crawford and Sheehan, 1985) which might have utilized almost entire pool of protein and AA in HL and reduced their levels (Fig. 2,3). By d-14, the infected grubs reach death phase (Bedford, 1981; Mohan *et al.* 1985) which would derange tissues completely and release virions and tissue contents and steeply increase protein level beyond that of healthy grubs by d-16 PI (Fig. 2) (Shigematsu and Noguchi, 1969; Young and Scott, 1970, Payne, 1974, Kelly, 1976; Bedford, 1981; Mohan *et al.* 1985). Continuous degradation of protein by steady increase of protease activity from d-9 PI (Fig. 4) and complete blockage of host synthesis would increase the protein deficiency (Young and Scott, 1970; Shapiro and Ignoffo, 1971; Takei and Tamashiro, 1975). Sudden elevation of protease activity from d-9 PI (Fig.4) also favours high rate of cellular derangement and release of protease in HL. This would ultimately degrade protein (Fig. 2) randomly and increase AA level in diseased grubs on d-14 PI (Fig.3). The decline of AA and protease from d-14 PI (Fig. 3,4) might be due to complete degradation of various macromolecules by different degenerative activities of dying insects.

The increasing trends of protein and AA levels, and gradual increase of protease activity in healthy grubs upto d-14 PI (Fig. 2,3,4) are at par with normal metabolism of insects which tend to increase upto last instars and decline at prepupal stage (Wyatt, 1967; Van Der Geest and Borgsteede, 1969; Smilowitz, 1971; Wigglesworth, 1972; Takei and Tamashiro, 1975).

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