Protein Synthesis in Fat Body of *Trichoplusia ni* during the Course of a Nuclear Polyhedrosis Virus Infection

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Protein synthesis in fat body of cabbage looper, *Trichoplusia ni*, larvae was investigated during the course of a nuclear polyhedrosis virus infection. Fat body total protein concentration increased late in the disease due to a large increase in the saline-insoluble protein fraction. Incorporation of 14C-leucine into fat body proteins in vitro revealed a slight increase in synthesis of both retained and released proteins on the first day of the disease followed by a rapid decrease in protein synthesis. The rate of decrease was more rapid in released than in retained proteins.

Fat body soluble proteins of healthy larvae were separated into 22 bands by disc acrylamide gel electrophoresis. In diseased larvae, the concentration of major protein bands decreased during late stages of the disease. Electropherograms of the soluble fraction following 14C-leucine incorporation revealed most protein synthesized in the fifth instar of healthy larvae was in 2 zones close to the origin of the gels. These proteins were rapidly released following synthesis and accounted for all but trace amounts of the released protein. Synthesis of these major proteins decreased rapidly in diseased larvae after the first day as did synthesis of released proteins. Radioactivity in other areas of the gels was well maintained in retained proteins of diseased larvae until the fourth day when little radioactivity was present in any area of the gel.

INTRODUCTION

The fat body plays a major role in insect metabolism and is a site of nuclear polyhedrosis virus (NPV) infection in lepidopterous larvae. It is the primary site of synthesis and storage of proteins, including hemolymph proteins (Shigematsu, 1958; Locke and Collins, 1965, 1968; Chippendale and Kilby, 1969). Since viruses direct protein synthesis in infected cells to form viral products, an NPV infection of the fat body should alter synthesis and release of normal proteins during the course of disease. As defined by Price (1966), released protein is that protein which is synthesized by the fat body and released into the medium during the incubation period. Retained protein is the protein present in the fat body at the end of the incubation period. There are only a few reports on protein synthesis in NPV-infected tissues. Shigematsu and Takeshita (1958) reported an increase in fat body protein concentration late in the course of disease in NPV-infected *Bombyx mori*. Shigematsu and Noguchi (1969a,b) showed a rapid increase in protein synthesis in fat body and silk gland of NPV-infected *B. mori* and attributed the increase to synthesis of viral and polyhedral proteins.

Watanabe (1968, 1970) separated midgut proteins of cytoplasmic polyhedrosis virus-infected *B. mori* by disc acrylamide gel electrophoresis and reported a slight decrease in the concentration of some normal protein bands plus the appearance of a polyhedral protein band late in the disease. However, the effect of an NPV infection on protein synthesis of susceptible tissues has not been investigated by electrophoresis.

This investigation was conducted to determine the effect of NPV infection on protein synthesis in fat body of the cabbage
looper, *Trichoplusia ni*, throughout the course of infection, with emphasis on synthesis and release of normal fat body proteins.

**MATERIALS AND METHODS**

The virus used in this investigation was a single nucleocapsid per envelope-type NPV of *Trichoplusia ni*. The NPV was purified by the method of van der Geest (1968) as modified by Scott et al. (1971).

Early fifth-instar *T. ni* larvae were reared as described by Young and Scott (1970) and were infected by placing on a diet containing $1 \times 10^7$ polyhedral inclusion bodies per milliliter of diet. An equal number of control larvae was maintained on diet without virus.

Fat bodies were isolated at 24, 48, 72, and 96 hr after virus infection. Fat body from healthy larvae was obtained only at 24 and 48 hr as pupation occurred prior to the 72-hr analysis. The larvae were thoroughly washed in distilled water. The head and thorax were removed, the abdomen inverted, and the fat body isolated in cold 0.14 M NaCl. After adhering tissues were removed the fat body was washed twice in cold 0.14 M NaCl.

Fat body protein concentration was determined from combined tissue of 10 larvae. The tissue was homogenized in cold 0.14 M NaCl with a glass homogenizer and the volume increased to 3 ml. One milliliter of this homogenate was dried overnight at 100°C, and the dry weight was determined. Total fat body protein was determined from a second milliliter of the homogenate. The soluble fraction of fat body protein was determined from the third milliliter of homogenate following centrifugation at 20,000 g for 1 hr. Protein concentrations were determined by the biuret method of Gornall et al. (1949). The value for each determination was the mean of 8 replications of 10 larvae each and was expressed as micrograms of protein per milligram dry weight of fat body.

Fat body protein synthesis was investigated in vitro by transferring isolated fat bodies from 5 larvae to a round-bottomed flask containing 1 ml of Stevenson and Wyatt's (1962) Incubation Medium Mixture A at pH 7.0. All media contained streptomycin sulfate (25 μg/ml) and penicillin G (250 units/ml). The flask was equilibrated on a water bath for 5 min at 25°C, after which 1 μCi of uniformly labeled $^{14}$C-leucine was added and the flask was incubated, with gentle shaking, for 1 hr. After incubation, the flask contents were transferred to a centrifuge tube and the flask was rinsed with 0.5 ml of incubation medium. The tube was centrifuged at 3,000 g for 5 min to pellet the fat bodies. The supernatant containing released protein was removed with a pipette. Extraction of protein and determination of $^{14}$C-leucine incorporation into retained and released proteins were by the method of Price (1969). The protein fraction was dissolved in a biuret reagent and after measuring the protein content of the solution, 100-μl samples were applied to strips of Whatman No. 1 paper and dried. Each strip was placed in a glass scintillation vial and 17 ml of scintillant (0.4% PPO, 0.01% POPOP in xylene) was added to each vial. Radioactivity was measured over a 10-min period in a Nuclear Chicago Model 6800 scintillation counter with 51% counting efficiency. All counts were corrected for background activity. The value for each determination was the mean of 6 replications of 5 larvae each.

Soluble fat body protein fractions of healthy and infected larvae were subjected to disc electrophoresis on 7% acrylamide gels as described by Ornstein (1964). The sample gels contained 17 μl of the soluble fat body protein fraction per column. A buffer of 0.1 M Tris-borate, pH 9.5, was employed. The gels were run at 2 milliamperes/column and 4°C until the tracking dye reached the bottom of each tube.

Migration of specific fat body soluble proteins and released proteins was investigated by electrophoresis following in vitro in-
corporation of $^{14}$C-leucine. The released proteins and fat body soluble protein fraction were made 60% with sucrose and layered on gels at the rate of 75 $\mu$l and 25 $\mu$l per column, respectively. Following electrophoresis, the gels were sectioned into thicknesses of 2 mm. Gel slices were air dried for 12 hr and solubilized by the procedure of Tishler and Epstein (1968). They were counted in 11 ml of a toluene-based scintillation fluid (0.4% PPO, 0.01% POPOP, 9.0% Beckman solubilizer BBS-3 in toluene).

**Results**

Fat body protein concentration in NPV-infected larvae increased throughout the disease (Fig. 1). The increase in concentration was slight early in the disease and was similar to that of healthy larvae through the second day. Thereafter a major increase in protein concentration occurred in diseased larvae from 180 $\mu$g/mg on the second day to 260 $\mu$g/mg on the fourth day of infection. Healthy larvae pupated prior to the third day and were not investigated further; however, other workers have shown a decrease in fat body protein concentration in lepidopterous pupae (Shigematsu and Takeshita, 1958; Chippendale and Beck, 1967; Chippendale and Kilby, 1969). The soluble protein fraction of the fat body, which consists of those proteins in the supernatant following centrifugation of the fat body homogenate at 20,000 $g$ for 1 hr, showed little increase during the first 3 days of disease and decreased on the fourth day. Therefore, the increase in protein concentration late in the disease was due to the insoluble protein fraction.

The effect of virus infection on in vitro incorporation of $^{14}$C-leucine into fat body proteins is shown in Fig. 2. Major changes in protein synthesis occurred in diseased fat body, whereas little change occurred in syn-

![Fig. 1. The effect of NPV infection on the fat body protein concentration of fifth instar larvae. Solid lines, healthy larvae; broken lines, infected larvae. Vertical lines are standard errors of the means.](image-url)
Fig. 2. Changes in the protein synthesis rate of fat body in fifth instar larvae during the course of an NPV infection. Solid line, healthy; broken line, infected. Vertical lines are standard errors of the means.

thesis in healthy larvae prior to pupation. Protein synthesis in diseased larvae appeared slightly higher than in healthy larvae on the first day in both retained and released proteins. However, on the second day, protein synthesis declined in diseased larvae to a point lower than that of healthy larvae and the decline continued throughout the disease. The decrease in synthesis of retained proteins of the fat body was gradual from day 2 to day 4 but the decrease was much more rapid in released proteins, and synthesis was minimal by the 3rd day.

The electrophoretic pattern of the soluble fraction of retained fat body proteins of healthy larvae was similar for both days examined (Fig. 3). A total of 22 protein bands was present. Diseased larvae showed a decrease in the concentration of some protein bands late in the disease, particularly on the fourth day. This decrease was most pronounced in major bands near the origin of the gels. No new protein bands were detected at any time during the course of the disease.

Disc electrophoresis of the soluble fraction
of retained proteins labeled with $^{14}$C-leucine from healthy larvae showed much of the radioactivity to be present in the major bands of zones A and B (Fig. 4). Some protein synthesis also occurred in the proteins present in zones C and D, but little radioactivity was present in the minor protein bands of zone E. Bands in zones A and B accounted for all but trace amounts of the released proteins (Fig. 5). The NPV-infected fat body soluble retained proteins generally exhibited a pattern of radioactivity similar to that of healthy fat body early in the disease. However, the rate of $^{14}$C-leucine incorporation into proteins of each zone changed as the disease progressed. A sharp decrease in the rate of incorporation of radioactivity into the bands of zones A and B occurred from the 2nd through the 4th day of disease. The response was quite different in zones C and D as there appeared to be an increase on the second day of disease and only a slight decrease on the third day. Little protein synthesis occurred in any area of the gel on day 4. The pattern of released protein in diseased larvae was similar to healthy on the first day with rapid incorporation of radioactivity into the bands of zones A and B. However, there was a sharp decrease in radioactivity in these zones on the second day in diseased larvae and little $^{14}$C-labeled protein was released on the 3rd day.

**Discussion**

Results of this investigation showed that major changes occur in the synthesis of fat body proteins in larvae of *T. ni* during the course of a NPV infection and corroborated those reported for NPV infection of *B. mori* (Shigematsu and Takeshita, 1958; Shigematsu and Noguchi, 1969a, b). The rapid increase in fat body total protein concentration late in the disease was accounted for by the insoluble protein fraction. This increase in insoluble protein might indicate an accumulation of virion and polyhedral protein in the nucleus of infected cells during late stages of the disease.

The results of $^{14}$C-leucine incorporation showed a slight increase in protein synthesis of NPV-infected larvae on the first day, followed by a rapid and continuous decline in protein synthesis throughout the course of disease. The increase in protein synthesis on Day 1 occurred with both retained and released proteins and indicates stimulation of normal protein synthesis early in the disease. Synthesis of released proteins decreased rapidly after the first day and occurred almost entirely during the middle stages of disease on days 2 and 3. Since histological studies have shown virus multiplication to spread throughout the fat body during this period (Mathad et al., 1968; Vail and Hall, 1969), these results show synthesis of released proteins decreasing as the disease progresses in the infected tissue. A decrease in synthesis of released proteins also would account for the pronounced decrease in he-
molymph serum protein concentration in T. ni larvae during the latter stages of disease (Young and Lovell, 1971).

The ratio of retained to released proteins synthesized increased as the disease progressed, indicating a shift to virus-coded protein synthesis. Synthesis of virus-coded proteins in the cytoplasm of NPV-infected cells has been shown in B. mori using fluorescent antibody techniques (Krywienczyk,
Fig. 5. Radioactivity in electropherograms of fat body released proteins in fifth instar larvae during the course of an NPV infection. H, healthy; D, diseased. 1, 2, 3, 4, number of days larvae infected. An $R_f$ scale is at the bottom.

1963). However, disc electrophoresis of the soluble fraction of retained proteins did not reveal new protein bands in diseased larvae. Failure to show additional protein bands in the soluble fraction could be due to rapid migration of newly synthesized virus proteins to the nucleus which would prevent their accumulation in the cytoplasm as the disease progressed. The large number of normal fat body proteins would have made
it difficult to identify new proteins present in the cytoplasm in low concentration.

REFERENCES


