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Haemocyte Changes in Larvae of the Tobacco Caterpillar *Spodoptera litura* F.. Infected with a Nuclear-Polyhedrosis Virus

BY

ABRAHAM JACOB [1] and T. R. SUBRAMANIAM [2]

ABSTRACT

Haemocyte changes in the larvae of *Spodoptera litura* during the course of a nuclear polyhedrosis are described. Early fourth instar larvae were inoculated with 10^5 polyhedra each. The number of circulating haemocytes (THC) was significantly higher in infected larvae at 24 hour after inoculation. But at 48, 72, 96 and 120 hour following inoculation the THC was significantly lower than in the corresponding healthy larvae. Further the THC decreased progressively from 48 hour onwards in infected larvae as against a general increasing trend in healthy ones.

NTRODUCTION

Nuclear polyhedroses have been reorted to cause considerable changes oth qualitative and quantitative. in the lood cells of the infected larvae (Shairo, 1967, 1968; Shapiro *et al.*, 1969; Vittig, 1968; Kislev *et al.*, 1969). The urpose of the present paper is to report ne quantitative changes in haemocytes /hich occur in the larvae of *Spodoptera itura* infected with a nuclear-polyhedosis virus.

MATERIALS AND METHODS

Fouth instar larvae of *S. litura* within 6 to 8 hours after their third ecdysis were obtained from a disease free culture maintained in the laboratory on castor leaves. The room temperature varied between 22.3° and 29.6° C and relative humidity between 70 and 97 per cent. The larvae were inoculated with 10^5 polyhedral inclusion bodies each by a spot feeding technique. After the complete ingestion of the inoculum

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1. Assistant Entomologist, Department of Entomology, Agricultural College, Vellayani 695522, Kerala, India.

2. Professor and Head of the Department of Entomology, Agricultural College and Research Institute, Coimbatore 641003.

(6 hours approximately) the larvae were transferred to sterilised ½-pint plastic containers and provided with uncontaminated castor leaves. Another set of larvae treated similarly but without the virus inoculum served as control.

Haemolymph samples were drawn at 24, 48, 72, 96 and 120 hour after inoculation. Haemocytes of 10 larvae each from treated and control group were estimated at each interval. The larvae were immersed in hot water at 55 to 60°C for 2 to 3 minutes to prevent haemocyte clumping. Blood was withdrawn into a Thoma white cell pipette by cutting a proleg on the sixth abdominal segment. The drawn haemolymph was diluted 20 times with 2 per cent Vercene saline containing a trace of gentian violet. The pipette was shaken for several minutes and the first three drops discarded. The haemocytes were counted with a haemocytometer (improved double Neubauer ruling) at a magnification of 450X. The data are expressed as cells/microlitre (= THC). The statistical 't' analysis was used for comparing differences between means.

RESULTS AND DISCUSSION

The results are presented in Table 1. It will be seen that the average number of circulating haemocytes per microlitre in infected larvae was significantly higher at the end of 24 hour following inoculation. The entry of virus into the haemo lymph could have stimulated either mitotic division of haemocytes or the release of normally sessile cells. Kislev *et al.* (1969) observed that in *Spodoptera littoralis* certain types of blood cells are capable of phagocytosing free virus rods as well as polyhedra. A similar

TABLE 1

Average number of circulating haemocytes in Healthy and NPV infected larvae of *S. litura*

Post-inoclnation period in hour	Average number of circulating haemocytes/micron ± S.E. [a]		
	Healthy	Infected	% increase (+) or decrease (—) over healthy
24	17800 ± 529.34	25230 ± 1835.77	+ 41.74
48	20280 ± 350.90	18800 ± 484.19	— 7.30
72	18890 ± 617.76	15420 ± 653.73	— 18.37
96	33710 ± 1371.05	11210 ± 610.05	— 66.74
120	22180 ± 1668.16	9650 ± 613.88	— 56.49

[a] Average from 10 larvae per day

increase in THC has been reported by Beard (1945) in Japanese beetle grubs (*Popillia japonica*) infected with milky disease bacteria and by Sussman (1952) in silkworm (*Platysamia cecropia*) infected with the fungus *Aspergillus flavus*. However no such increase in THC was noted in the NPV infected wax moth, *Galleria mellonella* larvae (Shapiro, 1967) and the bollworm, *Heliothis zea* (Shapiro *et al.*, 1969).

At 48, 72, 96 and 120 hour after inoculation, infected larvae had significantly lower number of haemocytes compared to the control larvae. It is also interesting to note that from 48 hour onwards the total haemocyte count decreasd progressively at subsequent intervals recording the minimum at 120 hour after inoculation. On the contrary, in healthy larvae, the THC showed an increase as the larvae advanced in age with apparant declines at 72 and 120 hour after the initiation of the test. At 24 and 72 hour, the healthy larvae were in the premoulting stages and at 120 hour they were nearing the prepupal stage. As in the case of healthy larvae, the infected ones were in the premoulting stage at 24 hour and underwent ecdysis within 48 hours after the inoculation. But they failed to undergo a second ecdysis which should normally occur before 96 hours after the initiation of the test. The conspicious reduction of haemocytes in the infected larvae could have been brought about by an interference in the normol mitotic division of haemocytes by the infection. Inclusion bodies were observed in the nuclei of the circulating haemocytes 72 hour after inoculation, thus indicating the chance of virus interference in the mitosis. Further, a larger number of haemocytes were found disintegrated towards the latter stages of the disease as a consequence of infection of the cells. Similar reduction of haemocytes occured in *G. mellonella* larvae from 10 days after infection with a nuclear-polyhedrosis virus (Shapiro, 1967) and in *H. zea* larvae after exposure for 3 days to a high dose of nuclear polyhydra (Shapiro *et al.*, 1969).

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