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Physiology of Infection by the Red-Pigmented Bacterium, *Serratia marcescens* Bizio in the Tobacco Caterpillar, *Spodoptera litura* F.

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Infection of *Serratia marcescens* Bizio, a red-pigmented bacterium in the tobacco caterpillar, *Spodoptera litura* F. caused an increase in total nitrogen, protein, DNA, RNA, carbohydrate and glycogen contents and decrease in total fat. An increase in magnesium and sodium, and decrease in calcium and potassium content were also noticed in the case of diseased caterpillars compared to healthy ones.

Occurrence and pathogenicity of *Serratia marcescens* Bizio have been reported in *Agrotis ypsilon* (Hf.) by Chattopadhyaya and Mukherjee (1955), in *Athalia proxima* Klug. by Bogawat *et al.* (1966) and in *Spodoptera (Prodenia) litura* F. by Pandey and Rangarajan (1967). Recently, Narayanan and Jayaraj (1974) observed the pathogenic effect of this bacterium under laboratory conditions on 26 species of Lepidopteran insects. However, no work has been done on the physiology of infection by *S. marcescens* in tobacco caterpillar, *S. litura*. Hence, attempt has been made to study the effect of *S. marcescens* on protein, fat, carbohydrate, nucleic acid and mineral metabolism of *S. litura*.

MATERIALS AND METHODS

The effect of *S. marcescens* was studied on the fourth instar larvae of *S. litura*. The castor leaves were dipped in 48 hr old culture raised in nutrient broth containing 16 million cell counts per ml, dried, and placed inside the petri-dish, and groups of 10-15 larvae were allowed. All estimations were done using fresh material of whole body homogenates, excepting for total nitrogen and minerals, which were estimated using dry materials.

Total nitrogen was estimated by micro-kjeldahl method. The estimation of carbohydrates and glycogen was made following the method of Crompton and Birt (1967). Groups of 5-10 fourth instar larvae were homogenized in 0.3 N perchloric acid at 0°C and the content of carbohydrates was estimated in the acid extract colorimetrically by phenol-sulphuric acid method of Dubois *et al.* (1956). Glycogen was precipitated from the acid extract (0.5 ml) by addition of saturated sodium sulphate solution (0.25 ml) and ethanol 70 per cent v/v concentration. The glycogen fractions were assayed by the phenol/sulphuric acid method. For the esti-

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mation of total fat, larvae were homogenized in chloroform-methanol 2:1 (v/v) mixture following the method of Folch *et al.* (1957), extracted at 60°C for 5 minutes (Orr, 1964), and partitioned against 3 per cent (w/v) aqueous sodium chloride. The chloroform phase was drawn off, dried and the lipid residue was dissolved in a minimum quantity of diethyl ether and transferred to a weighing bottle. Ether was allowed to evaporate completely and the dry lipid residue was weighed.

The preliminary separation of nucleic acids and protein from other fractions was done following the method of Orr (1964). Groups of fresh larvae were homogenized at 0-2°C with ice cold 0.4 N perchloric acid and extracted overnight at 5°C. Lipids were removed by using chloroform-methanol mixture. The dry tissue was suspended in 0.5 N perchloric acid at 90°C for 20 minutes. The suspension was centrifuged and the supernatant solution was analysed for nucleic acids. The residue was reserved for protein extraction. DNA was determined colorimetrically by the diphenylamine method of Burton (1956) using sodium salt of calf thymus gland DNA (BDH, England), as the standard. RNA was determined colorimetrically in the perchloric acid extract by the Orcinol method using yeast RNA (BDH, England) as the standard (Webb and Levy, 1958). Protein was extracted from the perchloric acid insoluble residue and assayed colorimetrically by the method of Lowry *et al.* (1951) using bovine albumin as the standard.

Calcium, magnesium, sodium and potassium were estimated in triple acid extract of dried larval material following the method of Jackson (1958). Sodium and potassium were estimated by flame photometry, and calcium and magnesium by titrimetry using eriochrome Black-T and murexide indicators.

RESULTS AND DISCUSSION

Protein metabolism: It is seen from the Table that the mean nitrogen content in diseased larvae (148.0 mg/g) was higher than that of healthy larvae (116.3 mg/g). In the healthy larvae the content increased with age from 97 to 132 mg/g. In the diseased larvae, the content, though remained more or less the same in the three post inoculation periods, was always higher than in the healthy ones. The most pronounced increase (58.8%) in nitrogen content was observed at 12 hr. after treatment. The increased nitrogen content of diseased larvae may be due to high proteolytic nature of the bacterium as suggested by Bucher (1961). Similar increase in total nitrogen had been observed in *Bombyx mori* L. (Tarasevich, 1952) and *S. litura* (Jacob, 1972) infected with nuclear polyhedrosis virus. There was a general increase in the protein content of the diseased larvae. The maximum content in the healthy insect was 4.4 mg/g at 48 hr whereas it was 6.4 mg/g in the diseased larvae (Table). The finding

TABLE. Effect of *Serratia marcescens* Bizio on the tobacco caterpillar, *Spodoptera litura* F.

Contents (mg/g)	Hours after treatment						Mean	
	12		24		48			
	H	I	H	I	H	I	H	I
Total N	97.0	154.0 (+58.8)	120.0	132.0 (+10.0)	132.0	158.0 (+19.7)	116.3	148.0 (+27.2)
Protein	3.9	4.5 (+15.4	4.2	4.5 (+7.1)	4.4	6.4 (+45.5)	4.2	5.0 (+20.9)
DNA	1.4	0.9 (−35.0)	1.1	1.4 (+26.4)	1.2	3.2 (+170.9)	1.2	1.8 (49.2)
RNA	2.3	2.7 (+17.7)	0.7	1.8 (+147.3)	2.8	2.2 (−21.4)	1.9	2.3 (+16.5)
Total fat	61.1	45.9 (−32.9)	44.3	39.8 (−10.1)	47.0	32.7 (−30.5)	50.8	39.5 (−22.3)
Total carbohydrate	7.4	4.6 (−38.2)	10.6	9.9 (−6.9)	9.5	20.6 (+116.8)	9.2	11.7 (+27.3)
Glycogen	2.6	4.2 (+63.7)	4.3	7.7 (+80.8)	5.6	2.3 (−59.8)	4.1	4.7 (+14.0)
Ca	5.0	6.0 (+20.0)	8.0	5.0 (−37.5)	8.0	8.0	7.0	6.3 (−9.6)
Mg	3.7	5.5 (+49.9)	2.9	3.0 (+5.9)	3.0	3.0	3.1	3.8 (+21.9)
Na	3.0	5.0 (+66.6)	4.0	5.0 (+25.0)	5.0	4.0 (−20.0)	4.0	4.7 (+16.5)
K	50.0	50.0	50.0	50.0	60.0	30.0 (−50.0)	53.3	43.3 (−18.8)

H = Healthy; I = Infected.

(Figures in parentheses represent % increase (+) or decrease (−) from healthy insects).

that the infected larvae of *S. litura* contained a higher level of total protein content corresponds to the observation on the silk worm, *B. mori* infected with nuclear polyhedrosis (Watanabe and Kobayashi, 1969). The 45.5 per cent increase in total protein observed in the infected *S. litura* larvae at 48 hr after treatment is probably due to the rapid multiplication of this potential pathogen in the blood as suggested by Bucher (1960).

The diseased larvae had higher amounts of DNA (Table) than the healthy ones. The maximum content of DNA was noticed at 12 hr stage which was decreased during the subsequent stages in the healthy insects. In the diseased larvae, there was a gradual increase in the DNA content from 0.9 mg/g at 12 hr after treatment to 1.4 mg/g at 24 hr and 3.2 mg/g at 48 hr after treatment. The quantity of RNA in the diseased

larvae was increased by 147.3 per cent at 24 hr after treatment over the corresponding stage of healthy insects. At 48 hr after treatment there was a decrease in the diseased larvae by 20.4 per cent. The decrease in the amount of DNA in the growing healthy larvae of *S. litura* is in agreement with the report of Niemierko *et al.* (1956) in *Galleria mellonella* L. Similar increase in DNA has been reported by Tarasevich (1952) and Yamafuji *et al.* (1954) in *B. mori* infected with nuclear polyhedrosis virus, and in RNA by Morris (1966). However, the exact significance of the changes noted in the total RNA and DNA of the septicaemia disorder in *S. litura* larvae infected with *S. marcescens* is not clear.

Lipid metabolism: The average fat content was higher (50.8 mg/g) in the healthy larvae than in the diseased ones (39 5 mg/g), as seen in Table. The content in the healthy insect had decreased at 24 hr after treatment and there was an increase in the subsequent observation. Similar trend in fat content was observed in *Hyalophora cecropia* L. by Gilbert and Schneiderman (1961). It is evident from the histopathological study (Unpublished data) that at the moribund stage 48 hr after infection, there was a perceptible qualitative difference in the fat body cells. Comparable alteration with qualitative and quantitative changes in the fat content was observed in the case of jaundice infected silk worm (Mamedniyazor *et al.*, 1966; and in *S. litura* infected with nuclear polyhedrosis virus (Jacob, 1972).

Carbohydrate metabolism: The diseased larvae had a higher level of total carbohydrates, the average content being 11.7 mg/g as against 9.2 mg/g in the case of healthy larvae (Table). There was an increase in the total carbohydrate content throughout the period in the diseased larvae whereas in the healthy the maximum was 10.6 mg/g at the 24 hr period. A maximum of 116.8 per cent increase in the content was observed at 48 hr after infection. The increase may be due to both hypoglycemic and hyperglycemic conditions as reported in the case of insects infected with nuclear polyhedrosis and granulosis by Darrenkuang *et al.* (1966), and Martignoni and Milstead (1964). The change may be attributed to the bacteria-induced metabolic alteration.

As the major fraction of carbohydrates in insects, glycogen showed a gradual increase in the healthy larvae from 2.6 mg/g at 12 hr to 5.6 mg/g at 48 hr. In the case of diseased insects there was an increase (4.2 mg/g) at 12 hr after treatment and the increase was pronounced (7.7 mg/g) at 24 hr after infection with *S. marcescens*, the percentage increase being 63.7 and 80.8 respectively (Table). On the other hand, there was a drastic reduction (59.8%) in the diseased larvae at 48 hr after treatment. It is noteworthy in this connection that the healthy larvae had completed a moulting by 48 hr, while the diseased ones did not undergo the corresponding moulting. Thus

even without a moult the glycogen content showed a decrease at 48 hr. Reports on the gradual increase in glycogen content with age of larvae have been reviewed by Rockstein (1960). Comparable changes in the glycogen metabolism have been demonstrated by Morris (1962) in the case of Western oak looper, *Lambdina fiscellaria somniaria* (Hulst.).

Mineral metabolism: There was a reduction in the calcium content of the diseased larvae (9.6%), when compared to the healthy (Table). The content was increased in the healthy insects from 5 mg/g at 12 hr to 8 mg/g at 24 and 48 hr periods. In the case of diseased larvae the calcium content was 6 mg/g at 12 hr after treatment which declined by 37.5 per cent at 24 hr after treatment. The average magnesium content in the healthy insect was 3.1 mg/g as against 3.8 mg/g in the diseased larvae (Table). The maximum amount in the diseased insect was observed at 12 hr after treatment (5.5 mg/g) and a constant level of 3.0 mg/g thereafter. There was an increase in the sodium content by 16.5 per cent in the diseased larvae, when compared to the healthy. Gradual increase in the content was observed in the case of healthy whereas in the diseased larvae there was a decrease at 48 hr after treatment (Table). Decrease in the content of potassium (18.8 per cent) was noted in the diseased larvae. No change in the quantity of potassium could be detected between healthy and diseased larvae at 12 and 24 hr after treatment, whereas at 48 hr after treatment the reduction was as high as 50 per cent.

The reduction in the content of potassium observed in the diseased larvae of *S. litura* is in conformity with the report of Akune (1951) in *B. mori* and Jacob (1972) in *S. litura* infected with nuclear polyhedrosis virus. Arseniev and Bromley (1951) reported that insufficient amount of potassium in the oak leaves favoured the outbreak of polyhedrosis in *Antheraea pernyi* B. larvae. The reasons for the decline of calcium and potassium in the diseased larvae now observed are obvious. However, in the light of findings of Arseniev and Bromley (1951) the present observation on calcium and potassium, perhaps, gives an indication that the minerals act as factors in increasing the susceptibility of larvae to bacterial infection.

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