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Sodium coupled nutrient transport in the midgut tissue of the silkworm, *Bombyx mori* L.

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Abstract. Fifth instar larvae of *Bombyx mori* were administered with additional Na^+ ion orally. The concentration of Na^+ increased and that of K^+ decreased significantly in the haemolymph within 24 h. There was no marked difference in the ionic concentration of the midgut cell while K^+ increased in the gut content. The protease activity increased significantly in gut lumen with a resultant increase in amino acid concentration, which showed a decrease in gut tissue and haemolymph. The protein content did not show significant change in gut lumen, while in gut tissue and haemolymph it decreased markedly. The results are discussed in relation to the Na^+-linked nutrient transport mechanism in the gut epithelium of *Bombyx mori*.

Keywords. *Bombyx mori*; midgut; nutrient transport; proteases.

1. Introduction

In insects, absorption of nutrients depends on 3 important factors, namely composition of the diet, functioning of nutritive enzymes and membrane permeability of intestinal epithelium (Treherne 1958; Primor and Zlotkin 1980; Harvey 1982). Composition of mulberry leaf (Ito and Kobayashi 1978) and the activity of digestive enzymes in *Bombyx mori*, have been studied in detail (Ito 1978; Sarangi 1986). Further, the food of phytophagous insects is rich in K^+ which moves passively down chemical gradient into haemolymph (Harvey *et al* 1975) and often the nutrients are absorbed from the intestinal lumen, much faster than can be explained. However, very little information is available regarding the effect of Na^+ on the activity of digestive enzymes and the absorption of nutrients by the gut of silkworms. Earlier studies on nutrient absorption in silkworm *B. mori* have confirmed the presence of carrier mediated transport (Sacchi and Giordana 1980) and also active transport (Sacchi *et al* 1981) mechanisms. In the present study, an attempt has been made to observe the influence of Na^+ on the activity of midgut protease and the absorption and transport of proteins, amino acids and ions by the gut of the silkworm *B. mori*.

2. Materials and methods

Bivoltine (NB_{18}) silkworm race was maintained under standard laboratory conditions at 25–28°C and a relative humidity of 70–90% on mulberry leaves (M5 variety). The fifth instar larvae were divided into two batches. Batch I served as control and was fed with the normal leaves. Pilot experiments showed that higher concentrations of NaCl was phagorepellent. Therefore 2% NaCl was sprayed on mulberry leaves and air dried before feeding to the experimental larvae (Batch II) on the 2nd day and all the observations were made after 24 h of feeding.

For ionic determination, the haemolymph was collected in a test tube by cutting the caudal horn of the silkworm larva and was diluted with distilled water (1:19 times). Then the midgut was excised and the gut tissue and gut content were separated. The gut content was centrifuged at 1000 *g* for 5 min and the supernatant was diluted with distilled water (1:10 times). The gut tissue was made to a 10% (w/v) homogenate in distilled water, centrifuged at 1000 *g* for 5 min and the supernatant was diluted with 0·6 N $HClO_4$ (1:1 times). Na^+ and K^+ were assayed by using systronics digital flame photometer.

For the enzyme studies, the midgut was excised along with the contents after freezing the animals for about 12 h at −20°C. The midgut tissue and the gut contents were separately taken for the measurements of protease activity. A 10% (w/v) homogenate of both the materials was prepared in ice-cold borate buffer (pH 11), centrifuged in an IEC refrigerated centrifuge at 1000 *g* for 15 min and the supernatant was used as the enzyme source. Protease activity was measured according to Eguchi and Iwamoto (1982) with slight modification that the pH was 11 instead of 11·2. Protein concentration was determined according to the method of Lowry *et al* (1951). Three ml of tissue homogenate or haemolymph were deproteinised by addition of 80% methanol, the supernatant was collected after centrifugation at 1000 *g* for 10 min and used for the estimation of free amino acids (Moore and Stein 1954). Four to five larvae were taken for each determination during each spraying and the average value of 4 determinations along with standard deviation is presented (figures 1–3). Student's '*t*' test was conducted to assess the level of significance.

3. Results

The larvae accepted the mulberry leaves sprayed with 2% NaCl. Extra sodium in the experimental larval diet resulted in the increase in Na^+ and decrease in K^+ concentrations in the haemolymph significantly ($P = < 0{\cdot}001$). Although the gut content and gut tissue in experimental larvae showed a change in ionic composition, it was not significant except for a notable increase ($P = < 0{\cdot}005$) in K^+ concentration in the gut content (figure 1).

Figure 2 shows the effect of Na^+ on total free amino acids and total proteins of the gut content, gut tissue and haemolymph of the fifth instar larva of *B. mori*. The

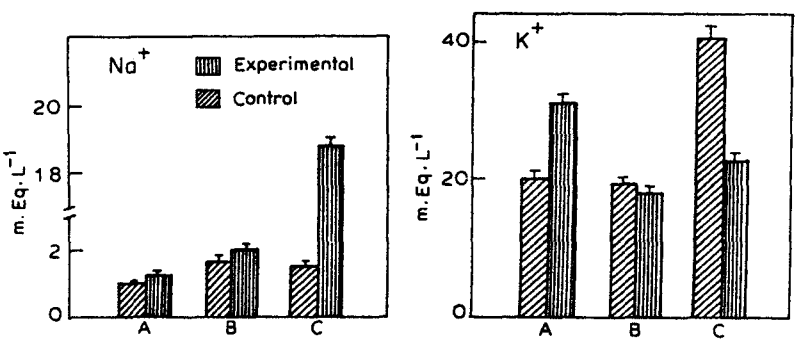

Figure 1. Effect of oral ingestion of Na^+ on the ionic composition of control and experimental larvae of *B. mori*. (A), Gut content; (B), gut tissue; (C), haemolymph.

concentration of total amino acids in the gut content increased 2·7-fold, while in both gut tissue and haemolymph it decreased following the administration of Na^+. The concentration of protein almost showed a similar trend as that of the amino acid content. However, the increase in the concentration of protein in gut content was not significant.

Figure 3 shows the activity of protease in the gut content and gut tissue of the fifth instar silkworm larva. In general, the protease activity was found to be 8–9 times higher in gut content compared to gut tissue. Oral administration of Na^+, though slightly decreased the protease activity in gut tissue, had a significant ($P = < 0{\cdot}05$) influence on the enzyme activity by increasing its level in the gut content.

4. Discussion

Active transport mechanism has been postulated to account for Na^+ absorption and its concentration in the haemolymph of the silkworm *B. mori* (Sacchi *et al* 1981) and in other insects as well (Shaw 1955; Farmer *et al* 1981). Barret (1982) has shown that Na^+ uptake could be mediated by a linked Na^+/K^+ pump studied at basal or

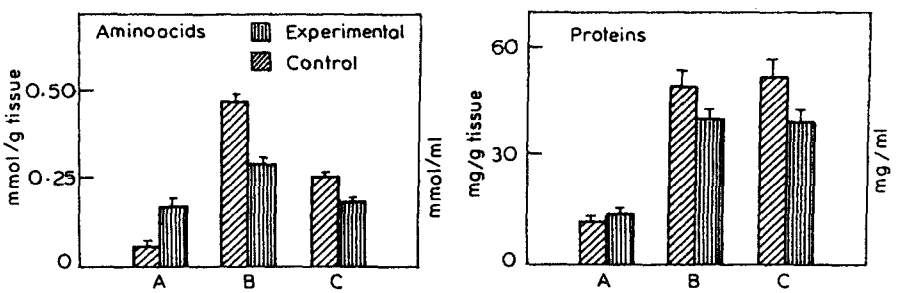

Figure 2. Effect of oral ingestion of Na^+ on protein and amino acid concentration of control and experimental larvae of *B. mori*. (A), Gut content; (B), gut tissue; (C), haemolymph.

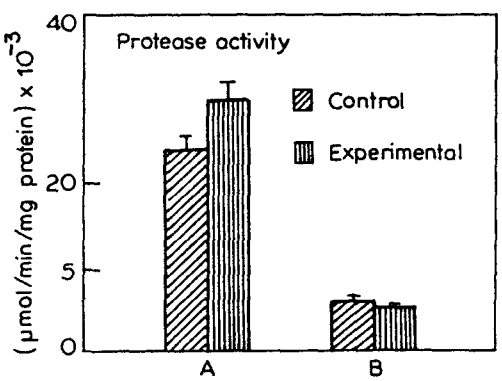

Figure 3. Effect of oral ingestion of Na^+ on midgut protease activity of control and experimental larvae of *B. mori*. (A), Gut content; (B), gut tissue.

lateral surface of the midgut cell. The present results show an increased Na^+ concentration in the haemolymph which might be due to its transport across the epithelial membrane into haemolymph. Further, to maintain the neutrality of haemolymph the K^+ concentration is brought down significantly. Relative lack of change in the ionic concentration of epithelial cells suggests that many substances cross the intestine without being accumulated in the cellular epithelium. The difference in the ionic composition in two compartments separated by midgut follows the same trend as reported earlier in *B. mori* (Giordana and Sacchi 1978). The increased K^+ concentration in gut content can be related to an active extrusion of K^+ across the goblet cells of epithelial membrane into the intestinal lumen in lepidopteran larvae as suggested by Harvey (1982). It is clear from the results that oral administration of Na^+ stimulates the release of midgut protease into the gut lumen. As a result, active hydrolysis of dietary proteins occurs which might partly contribute to the increase in the amino acid concentration in the gut content. But interestingly the concentration of proteins in the gut content does not show a significant change. As, the general food consumption did not show any apparent change in the experimental set, this requires further investigation to draw a conclusion that the total nitrogen uptake (proteins and free amino acids) by the larva increases under the influence of Na^+. It has been reported that addition of amino acids into the lumen increased the trans-epithelial potential difference in silkworms (Giordana and Sacchi 1978) and co-transport of some of the amino acids in the presence of K^+ or Na^+ is observed from lumen to the gut tissue (Giordana *et al* 1982). Thus an increased accumulation of amino acid in gut lumen as a result of Na^+ administration, might influence the transport of additional amount of amino acids across the epithelial membrane of the gut. Further, the decrease in the concentration of proteins and amino acids in the gut tissue and the haemolymph of the experimental larvae indicates that there exist a possible Na^+ mediated transport mechanism that facilitates the transport of nutrients from the gut tissue possibly to other tissues like silk gland and fat body during the fifth instar larval development of the silkworm, *B. mori*.

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