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THE ROLE OF CHOLESTEROL IN HAEMOLYMPH CATION PATTERN OF THE LAST LARVAL INSTAR OF THE MULBERRY SILKWORM *Bombyx mori* (L.)

By

M. M. ESSAWY
Plant Protection Dept., Fac. of Agriculture, Tanta Univ., Kafr El-Sheikh, Egypt.

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ABSTRACT

Changes in the concentration of cations in the haemolymph of silkworm *B. mori* were studied in both cholesterol-treated and normal larvae.

1- Sodium concentration in the haemolymph of treated larvae was lower than that of control.

2- The potassium level in the haemolymph of control larvae decreased during the obligatory feeding period. Then, from the 5th day to the prepupal stage, no change was observed. In treated larvae, during the first 7 days, potassium concentration was significantly lower than that of control. While, from day 8 and until pupation, potassium level was significantly higher than that of control larvae.

3- Na^{+}/K^{+} ratio in control larvae was lower than one. In treated larvae, during the first 7 days, the ratio was more than one. From day 8 up to pupation the ratio remained less than one and lower than that of control.

4- The calcium level was higher in treated larvae than that of control.

5- The haemolymph magnesium concentration increased significantly during the obligatory feeding period in treated larvae and reached (218.82 ± 9.8 mg/100 ml) at the 4th day. While, during the last three days, it was significantly higher in control larvae.

6- Ca^{+2}/Mg^{+2} ratio in control and treated larvae was lower than one. In treated larvae, the ratio was higher than that of control.

INTRODUCTION

Various forms of stress such as starvation (Jutsum et al., 1975 ; Lim and Lee, 1981), low temperature (Somme,1966), high temperature (Cohen and Patana, 1982) and desiccation (Edney, 1968) profoundly affect concentration of insect blood constituents. One aspect that has shown response to stress is cationic composition of the haemolymph (Cohen and Patana, 1982). However, cationic concentration is not only affected by stress, but also by changes during the developmental process (Florkin and Jeuniaux, 1974; Shimizu,1982; Essawy, 1985).

Lepidopterous larvae that feed on plant materials may witness a variety of host plant scarcity. Dietary inadequacy (Collett, 1976) and dietary condition (Jungreis et al., 1973; Shimizu, 1982) significantly modify the haemolymph cation pattern. Cation concentration in the silkworm haemolymph is important because the cations control the insect nervous system (Weevers, 1966 and Miyazaki, 1980) and its biochemical condition (Jungreis et al., 1974). Both the nervous system (Verrett and Mills,1976) and the endocrine system (Mordue, 1970; Hill and Izatt, 1974; Sroka and Barth, 1976) directly affect the concentration of most blood constituents.

After the treatment of B. mori larvae with cholesterol, silk production and food consumption were increased (Idriss et al., 1990). It is well known that sterols act as phagostimulants(Hamamura et al., 1962) in addition to its role as a structural component of membrane (Chen, 1984). Insects utilize sterol as their moulting hormone, and cholestrol is the obvious primary substrate for this hormone (Gilbert and Chino, 1974).

In the present study, variation of Na^{+}, K^{+}, Ca^{+2} and Mg^{+2} concentrations in the haemolymph of the last larval instar of B. mori were studied in both normal larvae, and larvae treated with a phagostimulant (cholesterol).

MATERIALS AND METHODS

Larvae of mulberry silkworm, Bombyx mori L. (Lep. Bombycidae) were used during the present experiments.Larvae of the Korean hybrid 155 x 156 were reared under laboratory conditions of 24 $\pm$ 1°C and 70-75% relative humidity. Cholesterol which has been produced by (Sigma St. Louis, MO, USA) was used.

All experiments were carried out on the last larval instar. A dose of fifty microgram of cholesterol dissolved in 2 ul of acetone was topically applied to the larval pronotum at 0$\pm$6 h. Each control larva was treated with 2 ul acetone, (Idriss et al., 1990)

According to Calvez, 1981 and Shimizu, 1982. The last larval instar has been divided into several periods : (i) The feeding period, which is divided into two periods, obligatory feeding period (from newly 5th instar larvae to 4-days old larvae) and facultative feeding period (4-days old larvae to 10-days old larvae), (ii) Mature larvae showing wandering behavior (10 days old larvae). (iii) Mature larvae which have just evacuated its midgut content (11 days old larvae). (iv) Early spinning period (12 days old larvae). (v) Middle spinning period (13 days old larvae). (vi) Prepupal stage (day 14) and (vii) new pupal stage at day 15.

Haemolymph of silkworm larvae was collected by cutting one of the abdominal legs, and that of pupae by puncturing the abdomen. After acid digestion with 0.1N HCl as used by(Dawczynski et al., 1980), each of the samples was analysed for Na^{+},

K^+, Ca^{+2} and Mg^{+2} with a Pye-Unicam Model 2800 Atomic Absorption Spectrophotometer apparatus. Statistical analysis of the data was performed using studient's t-test and P values <0.05 were considered significant.

RESULTS

Results of the quantitative changes of sodium in the haemolymph of control silkworm are shown in Fig.1a). Sodium concentration shows two peaks at feeding period. The first peak (65.35 ± 7.54 mg/100 ml) was noted during the obligatory feeding period (at the 2nd day) and the second peak (54.77 ± 12.75 mg/100 ml), was noted at the facultative feeding period (at the 8th day). This peak of the 8th day 8 was statistically significant. Then, a decline of Na^+ cation was noted, however, Na^+ cation concentration began to increase again at the prepupal stage, and rose up to about 27.4 ± 4.9 mg/100 ml.

In the haemolymph of treated larvae sodium cation showed a peak at the 2nd and 3rd days (Fig.1b). No significant difference between the two peaks of the 2nd day in control and treated larvae. A sharp and significant decrease at the 4th day was recorded and remained at a constant level up to the new pupal stage. The sodium concentrations in the haemolymph) of treated larvae were less than that of control.

Potassium concentration in the haemolymph showed a sharp decrease after ecdysis to the fifth instar larvae. This loss of K^+ continued until the fifth day of feeding period. Potassium concentration in the haemolymph of newly 5th instar larvae was 155.9 ± 21.8 mg/100 ml. While it was 43.68 ± 13.42 mg/100 ml in 5 days old larvae. Then, between the 6th day to the 12th day haemolymph K^+ stayed at rather constant level ranged from 53.76 ± 22 to 65.94 ± 12.29 mg/100 ml (Fig. 2a). A small increase which was

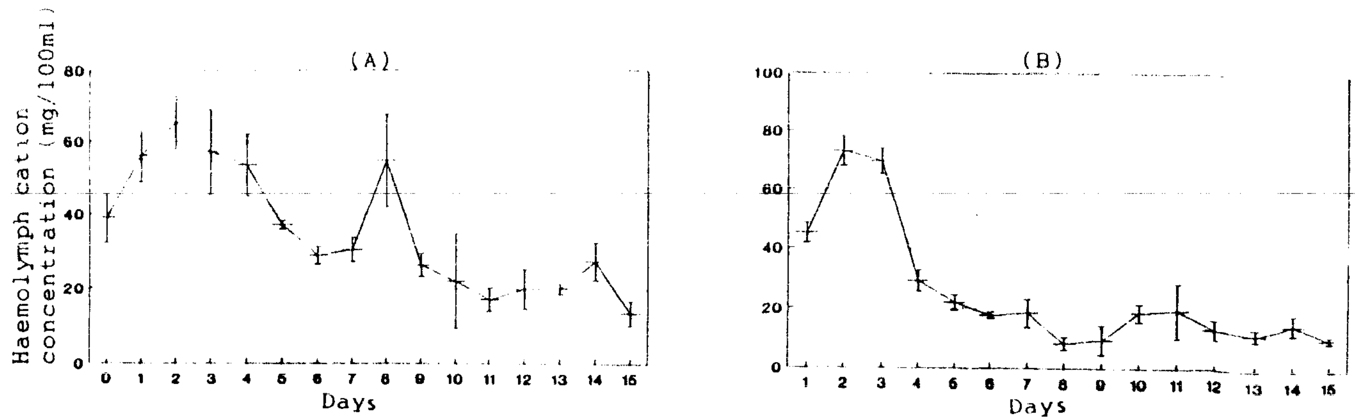

Fig (1): Variation of sodium concentration in the haemolymph of the silk worm larvae during the larval and larval pupal development. (A) Control larvae. (B) Treated larvae Mean ± SE.

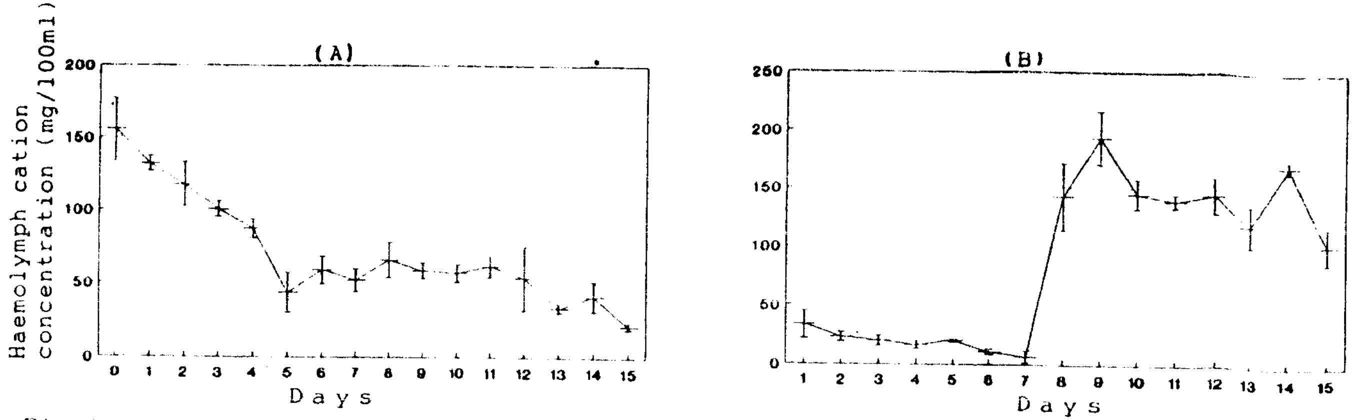

Fig (2): Variation of potassium concentration in the haemolymph of the silk worm larvae during the larval and larval pupal development. (A) Control larvae. (B) Treated larvae. Mean ± SE.

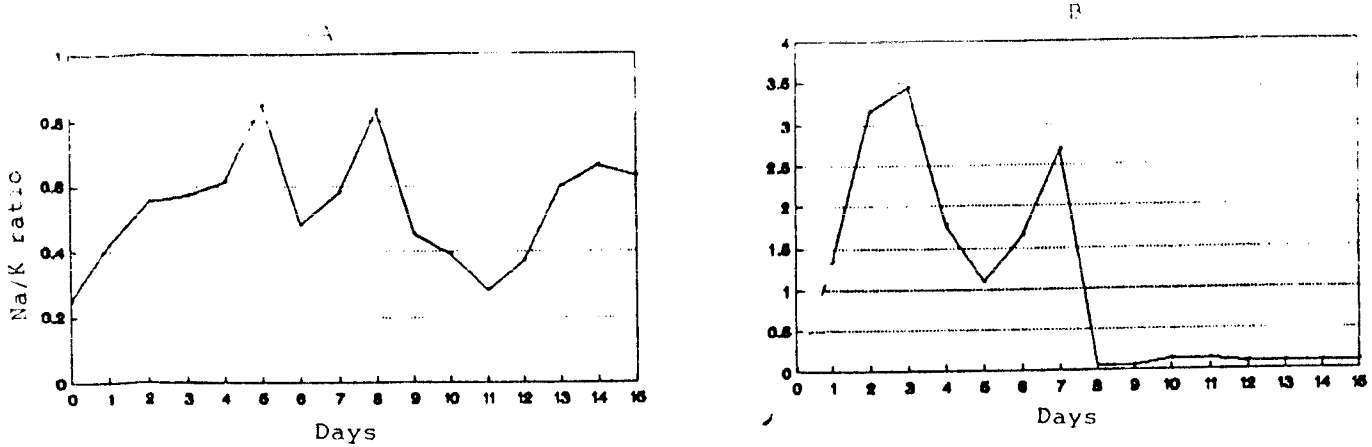

Fig (3): Na/K ratio in the haemolymph of the silkworm larvae during the larval pupal development (A) Control larvae (B) Treated larvae

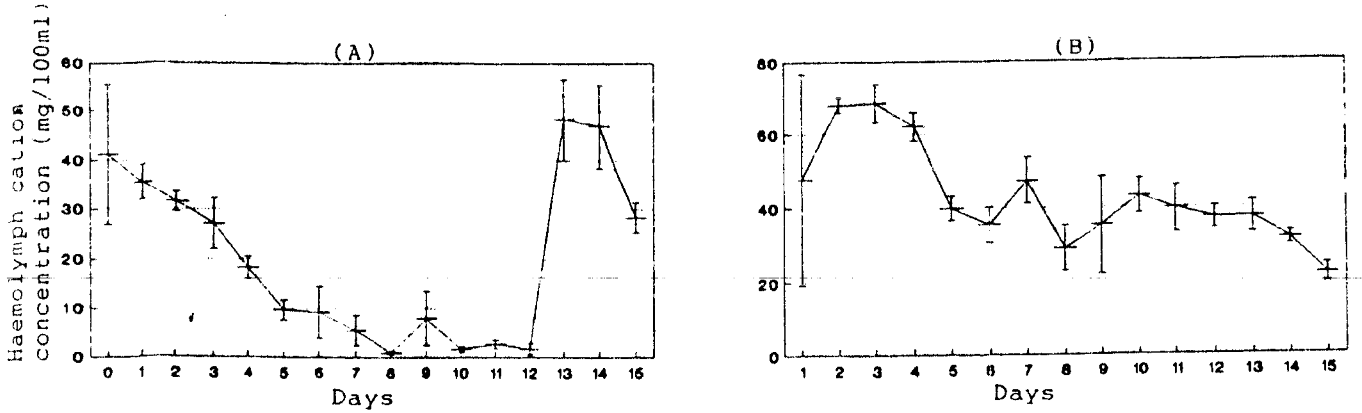

Fig. (4): Variation of calcium concentration in the haemolymph of the silk worm larvae during the larval and larval pupal development (A) Control (B) Treated larvae. Mean $\pm$ SE.

not significant at the prepupal stage was observed. A minimum level of K^+ was noted in the haemolymph of the new pupal stage (21.42$\pm$2.49 mg 100 ml).

Potassium cation in haemolymph of treated larvae showed a sharp decrease during the first 24 hours (from 155.9 $\pm$ 21.5 mg/100 ml to 33.4 $\pm$ 11.73 mg/100 ml). Then, potassium slightly decreased again up to the 7th day. However, in the last two days of facultative feeding period a high significant increase was observed. As a result, a slight decrease was noted until the 13th day (middle spinning stage). Another significant peak was noted at the prepupal stage (Fig. 2b). From this data, during the first 7 days, potassium cation concentrations in control and treated larvae exhibited the same trend. But in control larvae, K^+ concentration was significantly higher than that of treated larvae. While, from the 8th day and up to the pupation, in the treated larvae the K^+ haemolymph concentrations were significantly higher than that of control larvae.

The Na^+/K^+ ratio in control larvae ranged between 0.25 to 0.85, and remained constant at the typically low levels found in lepidopterous larvae as shown in (Fig.3a). This ratio reached its minimum at the end of the 11th day , which was equal to the ratio of Na^+/K^+ of newly 5th instar larvae. In treated larvae (Fig.3b), the Na^+/K^+ ratio reached its maximum at the 3rd day due to the increase of sodium and the decrease of potassium at this period. While at 7th day, another high ratio was noted which was due to minimal concentration of K^+ at this period. The ratios were about 3.44 and 2.68 at the 3rd and 7th days respectively. From the 8th day and until the pupation the Na^+/K^+ ratio remained constant and not more than 0.14. This was due to the elevated concentration of K^+, and to the constant and low level of Na^+.

Calcium concentration in haemolymph of control larvae showed a gradual decrease from the beginning of the last larval instar until the end of the fifth day. The level of Ca^{+2} remained constant at low level between the 5th day and until early spinning stage. At middle spinning stage Ca^{+2} augment about 29 fold (48.3 ± 8.36 mg/100 ml). Calcium concentration was constant at its high level during the prepupal stage, then decreased again at the new pupal stage.

In treated larvae calcium cation concentration was more higher than sodium, but they followed the same trend. Calcium presented a high level until the 4th day, then decreased significantly. Not significant peak was noted at the 7th day. From the 8th day to the prepupal stage Ca^{+2} concentration remained constant. In all studied times the Ca^{+2} concentration in the haemolymph of treated larvae were higher than that of control larvae except at the middle spinning stage to the new pupation.

In control larvae Mg^{+2} concentration in the haemolymph showed a constant state during the first four days of the feeding period. In the fifth day Mg^{+2} concentration decreased significantly. Then, it showed a significant increase in the 6th day (64.68 ± 19.6 mg/100 ml). Another peak was observed at the 8th day, followed by a gradual decrease until early spinning stage. At middle spinning stage a significant increase was recorded (101.01 ± 6.8 mg/100 ml). At the new pupal stage Mg^{+2} concentration reached to its minimal level (Fig.,5a).

In treated larvae haemolymph Mg^{+2} concentration exhibited a sharp and significant increase during the first four days of the feeding period, with a peak at the 4th day (218.82 ± 9.8 mg /100 ml). From the 4th day and up to the pupation a sharp and significant decrease was observed, and Mg^{+2} concentration reached its minimum at the new

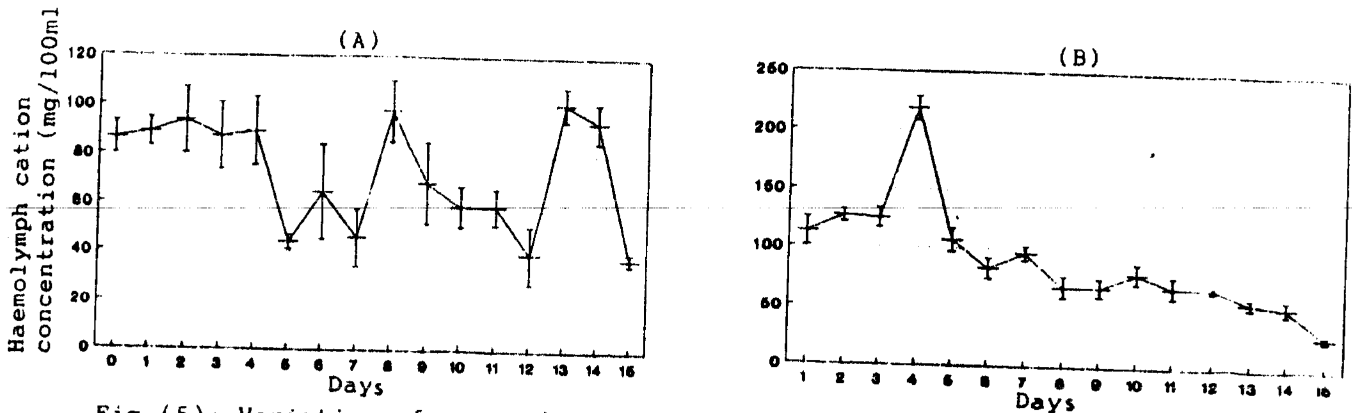

Fig (5): Variation of magnesium concentration in the haemolymph of the silkworm larvae during the larval and larval pupal development.
(A) Control larvae (B) Treated larvae. Mean $\pm$ SE.

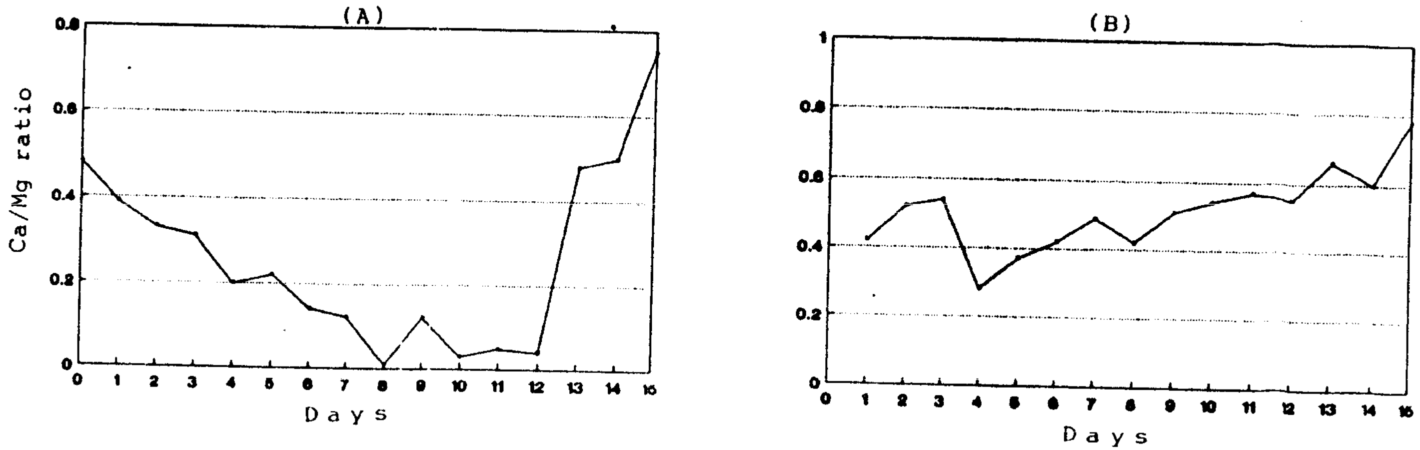

Fig (6): Ca/Mg ratio in the haemolymph of the silk worm larvae during the larval pupal development (A) Control larvae
(B) Treated larvae.

pupal stage. The increase of Mg^{+2} which was observed at spinning stage and prepupal stage in control larvae, had not been shown in treated larvae.

The Ca^{+2}/Mg^{+2} ratio was maintained at high level at the beginning and at the end of last larval instar development in control larvae (Fig. 6a). The ratio ranged between 0.01 to 0.75 and remained fairly not constant and reached its maximum at the new pupal stage. In treated larvae, Ca^{+2}/Mg^{+2} ratio remained constant as shown in (Fig.6b) and did not present a sharp modification. Also, in treated larvae, the ratio was higher in all studied times than that of control larvae (Fig. 6a,b).

DISCUSSION

Haemolymph ion concentrations are known to vary significantly with the time of feeding in an instar, the diet and dietary inadequacy, dietary condition and the rearing method (Pichon, 1970; Jungreis et al., 1973 ; Berneys and Chapman, 1974, Collett, 1976). Insects lack the ability to synthesize sterols from acetate and thus require them in the diet (Gilbert, 1967 ; Thompson et al., 1973). Hamamura et al, 1962 suggested that cholestrol may act as phagostimulant. Also, Ritter and Nes, 1981 noted that cholestrol supported the complete development of the insect.

From the present data haemolymph cation concentration was characterized by high level of magnesium and potassium in control larvae, and low level of calcium and sodium concentration. This data is in agreement with the results obtained by Florkin and Jeuniaux (1974), Dawczynski et al.(1980), Shimizu (1982) and Terra et al,(1982).

Sodium cation concentration in control larvae was higher than that of treated one. Stress conditions had little effect upon haemolymph sodium cation (Cohen and Patana,1982). A negative correlation between temperature and ability of Malpighian tubules to remove Na^+ from the haemolymph (Anstee et al. 1979). In the present observations a positive correlation was noted between the treatment with cholesterol and the decrease of sodium concentration in the haemolymph.

Shimizu (1982) noted a constant level of K^+ during feeding stage and the mature stage in silkworm, followed by increase at spinning stage and until pupation. This author explained these results to be due to the decrease of the haemolymph volume. In the present study, Potassium in control larvae, showed a significant decrease during the obligatory feeding period, while in facultative feeding period it remained constant. A decline and restoration of K^+ level in the cecropia haemolymph during larval-pupal transformation was observed (Jungries, 1978). In contrast to these results, treated larvae with cholestrol showed a low constant level during the obligatory and middle of facultative feeding period. Then, significant increase in K^+ concentration was noted at the 8th day and it was higher than that of control until the pupation, with a small decrease during spinning stage.

The Na^+/K^+ ratio in control larvae was less than one. While, in treated larvae the ratio ranged between 1.09 to 3.44 during the first 7 day, then decrease less than that of control. In lepidoptera(Chapman,1972) noted that Na^+/K^+ ratio may be lower than one, this ratio reduced the amount of regulation necessary to maintain a more or less constant level in the blood. Kimelberg and Papahadjopoulos,(1978) noted that cholesterol affect the activities of membrane associated enzymes such as Na^+, K^+ dependent ATPase.

In control larvae and during the first 4 days haemolymph Mg^{+2} concentration showed a high constant level, followed by a decline, then, two peaks appeared at the 6th and 8th days. Namouff and Jeuniaux, (1970) and Florkin and Jeuniaux (1974) noted a fall in haemolymph Mg^{+2} during the ontogeny. In the present data haemolymph Mg^{+2} showed a sharp increase at middle spinning stage followed by a significant decrease at new pupal stage. Concerning the elimination of the excess haemolymph magnesium, Kobayashi (1978) reported that in the silkworm, pupal midgut epithelium cells accumulated a large amount of Mg^{+2} as an inorganic form. This suggestion was supported by Shimizu (1982). In larvae treated with cholesterol, Mg^{+2} concentration increased significantly during the first four days than that of control larvae. This may explained by the increase of food intake due to the phagostimulant effect of cholesterol. Chapman (1972) noted that in phytophagous insects the concentration of Mg^{+2} in the blood is often relatively high due to the high level of Mg^{+2} in the diet, since it is a constituent of chlorophyll.

Calcium cation concentration in the haemolymph of control larvae showed a decrease and low level during the obligatory feeding period. A peak of calcium cation was noted at the middle of spinning period. While, in treated larvae calcium cation concentration showed a level which was more than that of control. In Spodoptera exigua Ca^{+2} concentrations were significantly affected by stress with decrease of this ion resulting from high temperature stress as well as starvation and desiccation (Cohen and Patana, 1982). In the silkworm B. mori, Shimizu (1982) reported that calcium concentration in the haemolymph of the larvae which were reared on mulberry leaves or an artificial diet was maintained at a constant level. Many functions were attributed to calcium cation in invertebrate and vertebrate. Since cellular activities are profoundly influenced by the haemolymph matrix, the chemical and osmotic composition of the haemolymph is very important to an insect's homeostasis (Schin and

Moore 1977). Smith and Gilbert (1989) reported that stimulation of ecdysone synthesis by prothoracicotropic hormone (PTTH), require extracellular calcium. Essawy (1985) showed that there was a strong positive correlation between extracellular calcium and ecdysone synthesis in the prothoracic gland. Extracellular Ca^{+2} can be completely expressed the activity of numerous vertebrates hormones (Steel and Paul, 1985), facilitates the activation of phosphorylase (McClure and Steel,1981) and control the initiation of DNA synthesis (Whitfield, 1980). Calcium affects the activation of calcium dependent adenylate cyclase (Martelly,1984). While, cholesterol affect adenylate cyclase (Klein _et al_.1978).

Vidair and Rubin (1982) noted that Ca^{+2} content, compartmentalization, and efflux rate are sensitive to small change in intracellular Mg^{+2}. Ca^{+2}/Mg^{+2} ratio in control and treated larvae in the present study were less than one, but the ratio in treated larvae was more than that in control larvae. According to the diagram of Szumiel (1983) it is the lowering on the intracellular free Ca^{+2}/Mg^{+2} ratio which may allow the initiation of DNA synthesis.

In conclusion, cholesterol as a phagostimulant, affect the cation pattern of the haemolymph in _Bombyx mori_ last instar larvae directly by increasing the food intake or indirectly by modifying the hormonal balance by increasing the available sterol.

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تأثير الكوليسترول على المحتوى الايونى للدم
فى العمر اليرقى الاخير لدودة الحرير

درس التغير فى تركيز الكاتيونات فى دم دودة الحرير سواء اليرقات المعامله بالكوليسترول أو اليرقات الغير معامله . وقد أوضحت الدراسه النتائج التاليه :

١- أن تركيز الصوديوم فى الدم كان أقل فى الحشرات المعاملة عن الحشرات الغير معاملة بالكوليسترول .

٢- ينخفض مستوى البوتاسيوم فى الدم فى اليرقات الغير معامله أثناء فترة التغذيه الاجباريه. ولم يكن هناك تغير معنوى فى مستوى البوتاسيوم من اليوم الخامس و حتى فترة ما قبل العذراء . أما فى الحشرات المعامله بالكوليسترول فان تركيز البوتاسيوم فى الايام السبعه الاولى من العمر اليرقى كان أقل معنويا عنه فى الحشرات الغير معامله . و من بداية اليوم الثامن و حتى طور العذراء فان مستوى البوتاسيوم فى الدم كان أعلى معنويا من تركيز البوتاسيوم فى الحشرات الغير معامله .

٣- كان معدل الصوديوم الى البوتاسيوم فى دم يرقات الحشرات الغير معامله أقل من واحد. بينما فى دم اليرقات المعامله أثناء السبعة أيام الاولى فان المعدل يكون أكبر من واحد، ولكن من اليوم الثامن و حتى التعذر فان المعدل يكون أقل من واحد و لكنه أقل من المعدل فى دم اليرقات الغير معامله .

٤- كان مستوى الكالسيوم أعلى فى اليرقات المعامله بالكوليسترول عنه فى دم اليرقات الغير معامله .

٥- يزداد تركيز الماغنسيوم فى الدم معنويا أثناء فترة التغذية الاجبارية فى اليرقات المعامله و يصل الى (٩،٨ ± ٢١٨،٨٢ ملليجرام / ١٠٠ مل) فى اليوم الرابع . و يظهر مستوى الماغنسيوم معنويا أعلى من المستوى فى اليرقات الغير معامله .

٦- و قد بينت الدراسه أن معدل الكالسيوم الى الماغنسيوم فى دم اليرقات المعامله و الغير معامله تكون أقل من واحد . و لكن فى اليرقات المعامله فان المعدل يكون أعلى منه فى اليرقات الغير معامله .