Changes in the Haemolymph Cationic Concentration in Larvae of the Silkworm *Bombyx mori* L. Exposed to Selected High Temperature Regimes

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**Abstract:** The effects of high temperature on cationic composition of haemolymph of the three selected races of the silkworm, *Bombyx mori*, viz., PM, NB4D2 and CSR2 during 4th moult and 5th instar larval development were investigated. Haemolymph cation levels increased significantly throughout the feeding period from 1st to 6th day in all the three races and showed a significant decrease on 7th day. Mg\(^{2+}\) and K\(^+\) ions were present at relatively higher levels followed by Ca\(^{2+}\) and Na\(^+\) both in the leaf and larval haemolymph. Among the races, haemolymph cation levels were higher in NB4D2 than CSR2 and PM. K\(^+\) levels of bivoltine races were about 30% higher than the multivoltine PM. The rates of elimination of cations were least during feeding stage than during non feeding stages resulting in higher cation levels during feeding stage. Despite increased feeding activity during active growth period, the cation levels showed significant drop in silkworm excreta suggesting that the cations are more completely absorbed during growth phase. When larvae were exposed to higher temperatures, monovalent cations like Na\(^+\) and K\(^+\) are hyper-regulated and divalent cations like Ca\(^{2+}\) and Mg\(^{2+}\) are hypo-regulated in order to prevent abnormal rise in the levels of haemolymph osmotic pressure. The lower levels of divalent cations in both haemolymph and excreta in larva haemolymph at higher temperatures suggest that the divalent cations are sequestered by intracellular fluids to counteract osmotic desiccation of somatic cells at higher temperatures.

**Key words:** Na\(^+\) - K\(^+\) - Ca\(^{2+}\) - Mg\(^{2+}\) - *Bombyx mori* - Mulberry leaf - Haemolymph - Temperature stress

**INTRODUCTION**

Haemolymph is a dynamic fluid tissue with close metabolic relationship with other tissues and organs. It plays an important role in excretion, defence, molting and metamorphosis [1]. Insect haemolymph plasma is characterized by a relatively high concentration of organic and inorganic electrolyte changes in response to diet and disease development and temperature stress [1]. The relative concentration of inorganic cations depends on the phylogenetic position of the insect group; more K\(^+\) and Mg\(^{2+}\) in haemolymph plasma being present in more evolved groups [2]. The osmotic and ionic composition of haemolymph plasma of silkworm undergoes changes due to increased evaporative water loss at high temperatures. Terrestrial insects are confronted with the problem of maintaining fluid and ionic composition of haemolymph plasma at levels best suited for the digestion and absorption of nutrients from the midgut and also to assist various physiological activities at different stages in their life cycle. The problem is compounded in monophagous insects which have to depend only on the leaf for its water and nutrient requirements. The mulberry leaf is the only source of water and electrolytes in a monophagous insect like the silkworm, *Bombyx mori*. The silkworm in the 5th instar is fed with coarse mulberry leaf which contains relatively less water and electrolytes than tender and medium leaves offered to larvae of 1st to 4th instars. The osmotic composition of the insect haemolymph is patterned after the mineral composition of the leaf of the host plant [3]. Ito, [4] observed that 28% of larval structures at different stages include absorbed minerals. The cations which contribute largely to ionic equilibrium in haemolymph plasma are Na\(^+\), K\(^+\), Mg\(^{2+}\) and Ca\(^{2+}\) which together with citrate contribute to 14% osmotic potential of haemolymph in a homopteran aphid, *Microsiphon albifrons* [2]. The osmotic pressure of haemolymph is influenced by the inorganic cation and anion like Na\(^+\) and Cl\(^{-}\) pump which are osmotic effectors in primitive pterygotes [5]. In case of Lepidopteran insects, the organic molecules or amino acids are the main osmolor effectors and have very low value of Na\(^+\) index and very
high values of $K^+$ and $Mg^{2+}$ indices [6]. Definite relative proportion of the $Na^+$, $K^+$, $Mg^{2+}$ and $Ca^{2+}$ are essential in the cell medium of insects [7]. The presence of these cations have been observed in the pupae of *Spilosoma lutea*, *Bombyx mori*, *Antharaea mylitta* [7], *Manduca sexta* and *Hyalophora cecropia* [8], *Morimus funereus* [9]. $K^+$ and $Mg^{2+}$ levels tend to be higher in the haemolymph of leaf eating caterpillars of lepidopterous insects reflecting the levels of the elements in plant tissues [10].

The ionic composition of haemolymph is maintained by balancing absorption of minerals through the midgut epithelium and excretion through Malpighian tubule. The operation of the homeostatic control mechanism of haemolymph cation composition is constrained in non-feeding stages of the insect life cycle like molting and metamorphosis due to restricted absorption of cations through the midgut epithelium. The excretion of ions is severely impaired during pupal stage due to retrogression of excretory system in pupa. Insects undergo considerable haemolymph volume changes at different stages of larval and pupal development [11] and in response to exposure to higher temperature and changes in cationic composition during such episodes have not been investigated in the mulberry silkworm, *Bombyx mori*. Haemolymph volume which protects the tissue water content is regulated by $Na^+$ and $K^+$ levels in desiccation resistant population of *Drosophila melanogaster* exposed to prolonged spells of desiccation [12]. The levels of the four major cations viz., $Na^+$, $K^+$, $Mg^{2+}$ and $Ca^{2+}$ were estimated in three selected races of the silkworm, *Bombyx mori* exposed to two selected high temperature regimes in order to understand the regulation of the ionic composition of haemolymph plasma under imposed thermal stress conditions.

**MATERIALS AND METHODS**

**Silkworm Rearing:** The silkworm breeds namely, Pure Mysore, NB4D2 and CSR2 were utilized in the present investigation to represent a tropical multivoltine, less productive bivoltine and highly productive bivoltine respectively. Disease free layings (DFLs) of the three races were brushed and reared as per the standard rearing technique of Dandin et al. [13]. Fresh mulberry leaves of V-1 variety were used for feeding the larvae.

**Temperature Treatments:** The optimum temperature recommended for rearing 5th instar larvae is $25\pm1^\circ C$ in all seasons. But the ambient temperatures in tropical countries range from 30-35°C in summer season and hence 31°C and 36°C were selected for high temperature treatment. The larvae were transferred one day before 4th moult into the chambers with three temperature zones viz., 25°C (control), 31°C and 36°C controlled with different thermostats representing optimal, high and very high temperatures respectively. The relative humidity (RH) was held constant at 70±5% at all the three temperatures.

**Haemolymph Collection:** Haemolymph was collected in a pre-chilled test tube containing a few crystals of thiourea by cutting the first proleg of larva. The haemolymph was collected from the 4th moult, post moult, feeding and spinning larval stages of control and temperature treated batches, and centrifuged at 3000g for 10 min at 4°C. The supernatants were used in the cation estimations.

**Estimation of Cations:** To 1ml of haemolymph, 10ml of diacid 9:4 (nitric acid and perchloric acid) was added. Similarly, known quantity of dried mulberry leaf powder and excreta were digested using digestion chamber (Digester 1009) until a clear solution was obtained. The solution was then cooled and the volume was made upto 20ml with double distilled water. The solution was filtered through Whatman No.1 filter paper. The cations were estimated from the aliquots of filtrate. Sodium and potassium ions were estimated by using Flame photometer (Elicol CL 360) while calcium and magnesium ions were estimated by using atomic absorption spectrophotometer (GBC 932 Plus).

**Statistical Analysis:** Analysis of variance (ANOVA) was used to test the significance of differences between the mean values of six independent observations of $Na^+$, $K^+$, $Mg^{2+}$ and $Ca^{2+}$ levels in the haemolymph of silkworm larvae. Tukey’s [14] multiple comparison tests were used to find significance of differences between the races, treatments and day wise changes. Differences were considered significant at $P < 0.05$.

**RESULTS**

The levels of $Na^+$, $K^+$, $Ca^{2+}$ and $Mg^{2+}$ in the haemolymph and silkworm excreta showed significant changes in all the three races at different stages of larval development and in response to exposure to higher temperatures (Fig. 1-8).

**Haemolymph Sodium Concentration:** The mean levels of $Na^+$ in larval haemolymph were higher in NB4D2
Fig. 1: Sodium levels in the larval haemolymph of the silkworm races reared at 25°C (Control), 31°C and 36°C. Each value is the mean±SD of 6 separate observations.

Fig. 2: Potassium levels in the larval haemolymph of the silkworm races reared at 25°C (Control), 31°C and 36°C. Each value is the mean±SD of 6 separate observations.

Fig. 3: Calcium levels in the larval haemolymph of the silkworm races reared at 25°C (Control), 31°C and 36°C. Each value is the mean±SD of 6 separate observations.
Fig. 4: Magnesium levels in the larval haemolymph of the silkworm races reared at 25°C (Control), 31°C and 36°C. Each value is the mean±SD of 6 separate observations.

Fig. 5: Sodium levels in the excreta of the silkworm races reared at 25°C (Control), 31°C and 36°C. Each value is the mean±SD of 6 separate observations.

Fig. 6: Potassium levels in the excreta of the silkworm races reared at 25°C (Control), 31°C and 36°C. Each value is the mean±SD of 6 separate observations.
Fig. 7: Calcium levels in the excreta of the silkworm races reared at 25°C (Control), 31°C and 36°C. Each value is the mean±SD of 6 separate observations.

Fig. 8: Magnesium levels in the excreta of the silkworm races reared at 25°C (Control), 31°C and 36°C. Each value is the mean±SD of 6 separate observations.

Table 1: Summary of ANOVA showing the effect of high temperature on haemolymph cation levels and silkworm excreta of the selected races of the silkworm, *Bombyx mori* L.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Cationic composition of mulberry leaf (meq/kg)</th>
<th>Cationic composition of haemolymph (meq/liter)</th>
<th>Cationic composition of silkworm excreta (meq/kg)</th>
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<tr>
<td>Mulberry leaf</td>
<td>Na⁺ 156.8±1.90&lt;sup&gt;a&lt;/sup&gt; K⁺ 424.8±10.3&lt;sup&gt;c&lt;/sup&gt; Ca²⁺ 192.2±2.70&lt;sup&gt;b&lt;/sup&gt; Mg²⁺ 669.2±26.8&lt;sup&gt;d&lt;/sup&gt;</td>
<td>PM 9.63±3.29&lt;sup&gt;a&lt;/sup&gt; NB4D2 11.1±2.99&lt;sup&gt;c&lt;/sup&gt; CSR2 10.5±2.82&lt;sup&gt;b&lt;/sup&gt;</td>
<td>PM 17.1±6.25&lt;sup&gt;c&lt;/sup&gt; NB4D2 14.8±5.79&lt;sup&gt;a&lt;/sup&gt; CSR2 15.6±5.18&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>F-test **</td>
<td>Haemolymph cationic composition (meq/liter)</td>
<td>**</td>
<td>Cationic composition of silkworm excreta (meq/kg)</td>
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<tr>
<td>Races</td>
<td>PM 9.63±3.29&lt;sup&gt;a&lt;/sup&gt; NB4D2 11.1±2.99&lt;sup&gt;c&lt;/sup&gt; CSR2 10.5±2.82&lt;sup&gt;b&lt;/sup&gt;</td>
<td>PM 17.1±6.25&lt;sup&gt;c&lt;/sup&gt; NB4D2 14.8±5.79&lt;sup&gt;a&lt;/sup&gt; CSR2 15.6±5.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>PM 17.1±6.25&lt;sup&gt;c&lt;/sup&gt; NB4D2 14.8±5.79&lt;sup&gt;a&lt;/sup&gt; CSR2 15.6±5.18&lt;sup&gt;b&lt;/sup&gt;</td>
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** Significant at 0.1% (P<0.001) Means with different superscripts are significantly different from each other for various races separately (as indicated by Turkey’s HSD)
The Na⁺ levels showed a significant decrease from 4th moult to 0-day (Fig. 1) and a significant increase was noticed upon resumption of feeding on 1st day and a consistent increase till 6th day in control batches and 5th day in temperature treated batches in all the three races. A significant decrease in Na⁺ levels was observed on 7th and 6th day in control and temperature treated batches respectively (Fig. 1). Upon exposure to higher temperature, the haemolymph Na⁺ levels increased in PM on all days of larval development and the increase observed were found to be relatively higher at 36°C than at 31°C. In NB4D2, the Na⁺ levels increased at 31°C on all days of larval development whereas at 36°C, a significant decrease in the haemolymph Na⁺ levels was noticed. In CSR2, the haemolymph Na⁺ levels showed a significant decrease at higher temperature and the levels of decrease were found to be relatively more at 36°C than at 31°C.

**Haemolymph Potassium Concentration:** The K⁺ levels were relatively higher than Na⁺ levels in the haemolymph plasma of all the three races (Table 1). The mean K⁺ levels were relatively higher in NB4D2 (44.5 meq/l) than CSR2 (43.4 meq/l) and PM (39.9 meq/l). A significant decrease in the K⁺ levels was noticed after 4th moult on 0-day in all the three races (Fig. 2). The larval K⁺ levels increased till 6th day of larval development in all the three races. Upon exposure to higher temperature, the larval haemolymph K⁺ levels showed a significant increase till 5th day in PM and the increase observed were found to be relatively higher at 36°C than at 31°C. In NB4D2, the K⁺ levels increased at 31°C whereas at 36°C, a significant decrease in the haemolymph K⁺ levels was observed. In CSR2, the haemolymph K⁺ levels showed a significant decrease at higher temperature and the levels of decrease were found to be relatively more at 36°C than at 31°C.

**Haemolymph Calcium Concentration:** The larvae of NB4D2 showed a relatively higher mean levels of Ca²⁺ (15.96 meq/l) than CSR2 (13.3 meq/l) and PM (11.9 meq/l) (Table 1). The Ca²⁺ levels showed a significant decrease from 4th moult to 0-day in control and temperature treated batches (Fig. 3). The Ca²⁺ levels regained upon resumption of feeding from 0-day to 6th and 5th day respectively in control and temperature treated batches of all the races and on the last day of larval development, a significant decrease in Ca²⁺ levels was noticed. Upon exposure to higher temperature, haemolymph Ca²⁺ levels showed a significant decrease throughout larval development in all the three races and the decrease observed were found to be relatively higher at 36°C than at 31°C.

**Haemolymph Magnesium Concentration:** The larvae of NB4D2 showed a relatively higher mean levels of Mg²⁺ (47.5 meq/l) than CSR2 (42.7 meq/l) and PM (35.1 meq/l) (Table 1). The haemolymph Mg²⁺ levels showed a significant drop in post moult larvae before 1st feeding but showed a significant increase in the feeding larvae from 1st day to 6th day. On 7th day when the larvae stop feeding, a significant decrease in haemolymph Mg²⁺ levels was noticed. A significant increase in haemolymph Mg²⁺ levels in PM was observed during active feeding period from 2nd to 5th day when the larvae were exposed to 31°C. Whereas, in bivoltine races, a significant decrease in the Mg²⁺ levels was observed at both 31°C and 36°C and the order of decrease was found to be more 36°C than at 31°C.

**Cationic Composition of Mulberry Leaf and Silkworm Excreta:** The levels of Mg²⁺ (669.2 meq/kg) in the mulberry leaf were relatively high followed by K⁺ (424.8 meq/kg), Ca²⁺ (192.2 meq/kg) and Na⁺ (156.8 meq/kg) (Table 1). The levels of cations in the excreta in PM, NB4D2 and CSR2 were in the order of K⁺>Mg²⁺>Ca²⁺>Na⁺ (Fig. 5-8). Among the races, the rates of excretion of cations (Na⁺, K⁺, Ca²⁺ and Mg²⁺) were found to be significantly higher in PM than CSR2 and NB4D2 (Table 1). The levels of cations (Na⁺, K⁺, Ca²⁺ and Mg²⁺) in silkworm excreta were relatively high during 4th moult, post moult (0-day) and spinning larvae (7th day). In the feeding larvae, a consistent decrease in the cation levels of Na⁺, K⁺, Ca²⁺ and Mg²⁺ in excreta from 1st day to 6th day was noticed in all the three races. At higher temperature, the levels of Na⁺, K⁺, Ca²⁺ and Mg²⁺ were relatively less than control batches. The decreases noticed in cation levels of Na⁺, K⁺, Ca²⁺ and Mg²⁺ of silkworm excreta were relatively more at 36°C than at 31°C in all the three races. Among the four cations, the levels of K⁺ in silkworm excreta were relatively more than the other three cations.

**DISCUSSION**

Cationic composition of insect haemolymph has been extensively studied in all the insect orders but data on different developmental stages in an insect species are not available [3]. The relative composition of cations in the haemolymph of *Bombyx mori* is similar to that of the mulberry leaf (Fig. 1-4 and Table 1). The cation composition of the haemolymph is dietetic, the relative
levels and proportions of cations in the diet being reflected in the composition of the haemolymph though it is wide departure from the other non-insect animal groups. K+ and Mg2+ levels were high in the leaves (Table 1) and the haemolymph of insect, which feed up upon such leaves has high K+ and Mg2+ levels (Fig. 2 and 4). Haemolymph cation levels increased significantly throughout the feeding period from 0-day to 6th day in all the three races. Diet, temperature and disease influence haemolymph cationic composition of an insect species [1].

The silkworm haemolymph plasma is characterized by low Na+ and very high K+ and Mg2+ concentrations and moderately high Ca2+ like the mulberry leaf. Weevers [15] reported similar haemolymph ionic composition in the Tasar silkworm, Antheraea mylitta. Na+ concentration in the mulberry leaf is relatively lower (Table 1) and to prevent Na+/K+ ratio falling too low, a Na+ pump, which transports Na+ ions from the lumen of the midgut to haemolymph is envisaged. The presence of such a Na+ pump is demonstrated in the Cecropia midgut, which was unaffected by high K+ [16]. Haemolymph cationic composition showed significant changes at the different physiologically distinct stages of growth and development viz., moult, post moult (before first feeding), feeding and spinning stages.

The levels of Na+ are significantly higher in the two-bivoltine races NB4D2 and CSR2 than multivoltine PM (Table 1); the difference could be attributed to the relatively higher levels of ingestion of bivoltine races than the multivoltine PM. In the case of K+ though the three factors ontogeny, mulberry leaf and silkworm race influence K+ levels of larval haemolymph, the relative effect of race was significantly more than the influence of the mulberry leaf and ontogeny. Efficient regulation of haemolymph K+ by active transport of K+ from the haemolymph via midgut has been suggested [17]. Very high haemolymph K+ levels also reduce the efficiency of Na+ pump [16]. But, K+ levels of bivoltine races are about 30% higher than the multivoltine PM. Higher osmolar concentration of haemolymph of bivoltine races provides a favorable passive absorption gradient of digested nutrients in the lumen to haemolymph across midgut epithelium. A close correlation between dietary and haemolymph Na+/K+ ratios has been observed in a number of insects [1]. The phytophagous insects have ratios less than one; carnivorous insects have ratios greater than one and ratios of omnivorous insects fall in between. Force-feeding of a selected cation leads only to a transitory increase in the cationic level in the haemolymph as insects can regulate the haemolymph cation levels.

The rates of absorption and excretion of cations appears to depend on the relative composition of cations in the mulberry leaf and their utility in functional homeostasis of silkworm larva. Mg2+ and K+ were present at a relatively higher levels followed by Ca2+ and Na+ both in the leaf and larval haemolymph. But, in feeding larvae K+ was excreted in significantly higher quantities than Mg2+ presumably to prevent Na+/K+ ratio dropping too low to affect neural function. Also, lower excretory rates of Na+ leading to relatively higher increase in the haemolymph levels of Na+ in feeding larvae helped in further improvement of Na+/K+ ratio. A negative correlation exists between the levels of cations in haemolymph and their levels in silkworm excreta at different larval stages of development. The haemolymph cation levels are held by controlled excretion of the cations (Fig. 5-8). Na+ levels in excreta of feeding larva are the least and K+ levels the highest. The rates of elimination of cations are least during feeding stage than during non feeding stages resulting in higher cation levels during feeding stage. Despite increased feeding activity during active growth period, the cation levels showed significant drop in silkworm excreta suggesting that the cations are more completely absorbed during growth phase. The haemolymph cation levels in spinning stage are brought to the levels existing in pre-feeding stages like moult and post moult by increased rates of elimination of haemolymph cations (Fig. 5-8). The cation excretion is relatively higher in spinning larvae than in feeding larvae leading to a drop in haemolymph cationic levels. The spinning larvae shrink in size and store large quantities of amorphous silk in the lumen of the silk gland. The immediate and proximate source of water for silk biosynthesis is haemolymph and the consequent volume reduction of haemolymph requires elimination of cations to maintain the osmotic pressure.

The haemolymph cation levels were relatively high during moult and less during post moult. An increase in haemolymph cation concentration was observed during IV moult in the larvae of the giant silk moth, Hyalophora cecropia [11]. The drop in the cationic levels from moult to post moult stage can be attributed to swift changes occurring in the fluid levels of haemolymph during moult cycle. Ziegler et al. [18] observed that the sudden increase in the haemolymph volume between late premoult and intramoult served to expand the cuticle during moult in the isopod, Ligia pallasii. The cations are present at relatively high concentration during feeding stage and appear to be determined by relative rates of their absorption from the midgut lumen and excretion through
the Malpighian tubule. The rates of absorption and excretion of cations appears to depend on the relative composition of cations in the mulberry leaf and their utility in functional homeostasis of silkworm larva. Mg\(^{2+}\) and K\(^+\) were present at a relatively higher levels followed by Ca\(^{2+}\) and Na\(^-\) both in the leaf and larval haemolymph. But, in feeding larvae K\(^+\) was excreted in significantly higher quantities than Mg\(^{2+}\) presumably to prevent Na\(^+\)/K\(^+\) ratio dropping too low to affect neural function. Also, lower excretory rates of Na\(^-\) leading to relatively higher increase in the haemolymph levels of Na\(^+\) in feeding larvae helped in further improvement of Na\(^+\)/K\(^+\) ratio. A negative correlation exists between the levels of cations in haemolymph and their levels in silkworm excreta at different larval stages of development. The haemolymph cation levels are held by controlled excretion of the cations (Fig. 5-8). Na\(^+\) levels in excreta of feeding larva are the least and K\(^+\) levels the highest. The rates of elimination of cations are least during feeding stage than during non feeding stages resulting in higher cation levels during feeding stage. Despite increased feeding activity during active growth period, the cation levels showed significant drop in silkworm excreta suggesting that the cations are more completely absorbed during growth phase. The haemolymph cation levels in spinning stage are brought to the levels existing in pre-feeding stages like moult and post moult by increased rates of elimination of haemolymph cations (Fig. 5-8). The cation excretion is relatively higher in spinning larvae than in feeding larvae leading to a drop in haemolymph cationic levels. The spinning larvae shrink in size and store large quantities of amorphous silk in the lumen of the silk gland. The immediate and proximate source of water for silk biosynthesis is haemolymph and the consequent volume reduction of haemolymph requires elimination of cations to maintain the osmotic pressure. Haemolymph Ca\(^{2+}\) levels do not change significantly during 5\(^{th}\) instar larval development though they are slightly higher in bivoltine races than multivoltine PM. The higher haemolymph Ca\(^{2+}\) has a significant role in silk secretion. Higher Ca\(^{2+}\) levels could be associated with higher productivity since Ca\(^{2+}\) is secreted as a constituent of silk. During moulting a significant decrease in haemolymph Ca\(^{2+}\) levels was observed. The role of Ca\(^{2+}\) in moulting cycle in insects has not been thoroughly investigated as in other invertebrates like crustaceans having a calcareous shell. Ziegler et al. [18] observed significant increase in the haemolymph Ca\(^{2+}\) concentration from the inter-moult to intra-moult and post-moult due to resorption of Ca\(^{2+}\) from the cuticle and external CaCO\(_3\) deposits. Higher haemolymph Mg\(^{2+}\) concentration was observed during moult in all the three races. A significant drop in the haemolymph Mg\(^{2+}\) concentration from moult to post-moult (Day 0) suggests that haemolymph Mg\(^{2+}\) concentration fall when dietary supply is cut off. A similar decrease in haemolymph Mg\(^{2+}\) levels in prepupal blood was reported [10].

When larval stages were exposed to higher temperature, monovalent cations like Na\(^+\) and K\(^+\) increased whereas divalent cations like Ca\(^{2+}\) and Mg\(^{2+}\) decreased, the per cent changes observed being more at 36\(^{\circ}\)C than at 31\(^{\circ}\)C. The haemolymph monovalent cations are hyper-regulated and divalent cations are hypo-regulated at higher temperature in their haemolymph levels. The haemolymph and excreta levels of monovalent cations show an inverse relationship suggesting regulation of their levels in haemolymph by controlled excretion. But, the haemolymph divalent cations were lower at higher temperatures despite lower rates of their excretion suggesting their incorporation by inter and intracellular fluids as a protection against osmotic desiccation at higher temperatures. Such bidirectional movement of cations between hemolymph and epidermis has been reported during molt cycle in Scylla serrata [19]. Cations like Na\(^+\), K\(^+\) and Ca\(^{2+}\) are bound to proteins in Galleria mellonella [20]. The contrasting change in the two kinds of cations and the higher degree of change at 36\(^{\circ}\)C than at 31\(^{\circ}\)C can be attributed to greater dehydration stress at the higher temperature of 36\(^{\circ}\)C. Nicolson [21] reported volume reduction in haemolymph without any apparent change in the osmotic and ionic concentration when the stick insect, Carausius morosus was exposed to prolonged dehydration stress.

REFERENCES