

THERMAL ACCLIMATION OF A CENTRAL NEURONE OF *HELIX ASPERSA*

I. EFFECTS OF TEMPERATURE ON ELECTROLYTE LEVELS IN THE HAEMOLYMPH

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SUMMARY

1. The chemical composition of the haemolymph of adult snails was significantly altered during thermal acclimation (from 19° to 4°, 12° and 30 °C). The osmotic concentration was highest in cold groups (4° and 12 °C) and lowest in warm-acclimated animals.
2. The concentration of sodium in the haemolymph was highest in warm- and cold-acclimated individuals and lowest at intermediate temperatures (12° and 19 °C), and showed significant correlation with [Mg].
3. Potassium concentrations were also increased during warm and cold (4 °C) acclimation, the increase being largest in warm-acclimated individuals.
4. The concentrations of calcium and magnesium ions showed relatively small changes during thermal acclimation. Calcium activity was found to be highest in warm and lowest in cold-acclimated snails.
5. Feeding increased the levels of potassium and calcium most significantly in the warm-acclimated group.
6. pH remained relatively constant in all groups.

INTRODUCTION

The process of thermal acclimation has been well reviewed by Lagerspetz (1974). Poikilotherms have been less studied than homeotherms, with the exception of fish (Prosser, 1967). Attention has been directed at the biochemical correlates of thermal acclimation and the behavioural changes induced by such acclimation (e.g. Prosser & Nagai, 1968). Little attempt has been made to pinpoint thermal acclimation of an identifiable cell, and thereby describe the precise electrophysiological changes wrought by thermal variation.

Terrestrial pulmonate molluscs present several advantages for the study of the cellular correlates of thermal acclimation. They possess large, easily identifiable neurones, and temperate species, at least, have the ability to withstand a fairly wide spectrum of external temperatures. Responses to high or low temperatures include aestivation and hibernation. Variations in the ionic composition of the haemolymph accompany such tolerance; Burton (1965, 1968*a, b*, 1971*a, b*) has shown that in the terrestrial molluscs, *Helix aspersa* and *Helix pomatia*, the ionic concentration of the

haemolymph shows seasonal fluctuations, and is strongly influenced by such factors as hydration, feeding and acid/base balance.

This paper is concerned with analyses of Na, K, Mg, Ca and Cl levels in the haemolymph of snails kept in each of four temperature regimes. The potential physiological importance of changes in the ionic contents of the haemolymph are manifold, ranging from the influence upon other 'compartments' of the snail (dart-sac, kidney and shell) to disruption of the integrity of excitable tissue. Subsequent papers (Langley, 1979*a, b*) correlate acclimation-induced changes in the haemolymph with acclimation-induced alterations of the spike characteristics of a large identifiable central neurone.

MATERIALS AND METHODS

Experimental animals

Adult specimens of *Helix aspersa*, with a shell diameter not less than 18 mm, were collected locally throughout the year. Animals were kept at one of four temperatures: 4 °C, 12 °C, a room temperature of 19 ± 2 °C, and 30 °C. Humidity was kept at 89% and day cycles (12 hour light, 12 dark) were kept constant except for the animals at room temperature where day length had natural variation. The high humidity rendered unlikely any difference in hydration, and thus difference in haemolymph concentration, between the four groups.

Animals were maintained under these conditions without being fed for a minimum of 4 weeks prior to being used in any experiment since acclimation takes a minimum of 3 weeks to become established. The effects of feeding 1–2 h before analysis of the haemolymph were also tested.

Analytical methods

Collection of 2.0–2.5 ml of haemolymph was carried out using a plastic syringe and silicone coated vials. Samples were collected from the mantle haemocoel through a hole drilled in the shell. All samples were spun prior to analysis.

Sodium and potassium concentrations were measured by emission spectrophotometry. Concentrations of calcium and magnesium were measured by absorption spectrophotometry (on a Pye Unicam SP-90A flame spectrophotometer). Tests indicated that the analyses were free of significant interference between ions in the haemolymph samples.

Calcium activity was determined with an Orion calcium-specific electrode (model 93-20) with a sample size of 0.5 ml. The electrode was connected to a Keithley electrometer, together with a 3 M-KCl reference electrode.

Chloride concentration was measured by electrometric titration against silver nitrate solution (0.01 N) according to the technique of Ramsay, Brown & Croghan (1955).

pH measurements were made on a Pye Unicam pH meter (model 292) using an Ingold miniature electrode capable of measuring samples of 2 ml.

Osmotic concentrations were measured using aliquots of haemolymph on a Biological Cryostat Osmometer (Clifton Technical Physics) in accordance with the technique of Frick & Sauer (1973).

Statistics

Comparisons of the data were made with the Student's *t*-test and the *F*-test, and differences held to be significant with $P < 0.01$.

RESULTS

Concentrations of Na, K, Ca, Mg and Cl

The concentrations of Na, K, Ca, Mg and Cl after 4 weeks of thermal acclimation are shown in Table 1. Concentrations of Ca or Mg were less altered by acclimation than were concentrations of K or Na. Feeding increased levels of K and Ca, but not those of either Na or Mg. The largest increase in K and Ca induced by feeding were found in the haemolymph of warm-acclimated snails (K rose from 6.89 to 9.1 mM, s.e. 0.32, and Ca from 4.94 to 5.42 mM; s.e. 0.29, $n = 12$).

Calcium activity was found to be increased by warm-acclimation and decreased by the cold (Table 1). Since the gross concentration of Ca was little affected by acclimation (Table 1), this result indicates an inverse relationship between acclimation temperature and the degree of calcium binding in the haemolymph.

Tests were made for correlation between elemental concentrations, pooling the data from all haemolymph samples, irrespective of acclimation conditions. The only significant correlation ($P < 0.01$) was between Na and Mg levels, such that:

$$[\text{Mg}] = 0.0796 [\text{Na}] - 1.215.$$

The haemolymph concentrations of *Helix aspersa* recorded by Burton (1968*a*) are also shown in Table 1 and are similar to those recorded here at the probably similar (laboratory) temperature.

Hydrogen-ion concentration

pH differed little between the four groups, with a mean value of 7.62 (s.e. 0.36, $n = 42$) for all groups (Table 1).

Osmotic concentration

The most significant change in osmotic pressure induced by acclimation (Table 1) was in the 12 °C group, where the concentration was 54 m-osmol greater than at the laboratory temperature (19 °C).

Feeding increased osmotic pressure most in warm-acclimated individuals, perhaps resulting from the increases in K and Ca reported above.

DISCUSSION

The ionic concentration of pulmonate haemolymph shows considerable variation in both laboratory and natural populations. The factors which influence the concentration of individual inorganic ions include acid-base balance, the recency of feeding, relative humidity and the season in which the animals are examined (Arvanitaki & Cardot, 1932; Burton, 1965, 1968*a-c*, 1970, 1971*a, b*; Chiarandini, 1964; Kostyuk, 1968; Roach, 1963; Sorokina & Zelenskaya, 1967; Trams *et al.* 1965). Sodium con-

Table 1. *Analysis of the ionic and osmotic concentrations of the haemolymph of snails from the four acclimation regimes. The probable error for the calcium activity measurements is ± 0.2 mM*

Data from Burton (1968*a*) is included as a comparison, although the temperature at which the snails were raised is not stated in his study.

Condition	Elemental concentrations in haemolymph (mM)						Osmotic pressure m-osmol	pH
	Na	K	Ca	Ca activity	Mg	Cl		
Cold-acclimated (4 °C)	69.10 \pm 2.50 (8)	4.75 \pm 0.16 (8)	4.85 \pm 0.03 (8)	1.60 \pm 0.03 (4)	2.47 \pm 0.17 (8)	77.00 \pm 0.26 (8)	222.79 \pm 6.63 (12)	7.73 \pm 0.35 (10)
Cold-acclimated (12 °C)	49.10 \pm 2.69 (6)	4.62 \pm 0.13 (8)	4.90 \pm 0.03 (8)	1.83 \pm 0.02 (6)	2.77 \pm 0.12 (8)	72.10 \pm 0.89 (8)	252.15 \pm 7.62 (12)	7.69 \pm 0.32 (10)
Laboratory (19 °C)	54.60 \pm 4.40 (8)	2.54 \pm 0.08 (8)	5.24 \pm 0.07 (5)	2.03 \pm 0.05 (4)	4.10 \pm 0.21 (8)	60.20 \pm 0.90 (8)	197.70 \pm 3.59 (10)	7.62 \pm 0.36 (12)
Warm-acclimated (30 °C)	76.21 \pm 3.70 (8)	6.87 \pm 0.26 (6)	4.94 \pm 0.04 (6)	2.10 \pm 0.16 (4)	3.55 \pm 0.41 (8)	74.20 \pm 1.16 (8)	184.0 \pm 4.74 (10)	7.50 \pm 0.21 (10)
(Burton, 1968 <i>a</i>) 19 °C	68.0 \pm 6 (29)	2.9 \pm 0.3 (29)	6.2 \pm 1.1 (27)	—	3.6 \pm 1.2 (27)	—	—	—

Mean values \pm s.e.; *n* values being in parentheses.

centration shows the largest fluctuation, for example from 46 to 129 m-mole/kg water in natural populations of *Helix pomatia* (Burton, 1965).

In the present study acclimation induced changes in the concentrations of Na and K, but not of Ca or Mg. The activity of calcium, however, was affected by acclimation. It seems probable that binding of the two divalent cations forms part of a complex homeostatic mechanism controlling their availability, as has been suggested by Burton (1976) for the Ca ion.

Correlation between sodium and magnesium concentrations, as was found in this study, has been found previously in hydrated *Helix aspersa* (Burton, 1968*a*) and fasted *Helix pomatia* (Burton, 1965). The two ions may have some combined role in stabilizing the osmotic pressure of the haemolymph. It appears that thermal extremes increase the movement of Na and K from tissue compartments into the haemolymph (cf. Gladwell, Bowler & Duncan, 1975).

There is one discrepancy between the present data and that reported previously for the haemolymph of *H. aspersa*. That is that Na concentration was found to increase at a high temperature, whereas it has previously been found to decrease (see Grainger, 1975).

Relative constancy of pH in the four groups indicates a strong control mechanism, possibly involving haemocyanin and the Ca²⁺ buffer system in the fashion suggested by Burton (1976).

The changes in osmotic concentration that were observed could at least in part be due to changes in the degree of ionization of electrolytes (as demonstrated by the calcium activity measurements) as well as due to changes in the gross concentrations of electrolytes or proteins.

From the experiments reported in this paper it appears that thermal acclimation in *Helix aspersa* results in major changes in Na and K levels. Since sodium and potassium are likely to be primary determinants of the electrical characteristics of excitable cells in *H. aspersa*, the effects of these concentration changes on electrophysiological parameters will be considered in subsequent papers (Langley, 1979*a, b*).

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