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THE COMPOSITION OF THE HAEMOLYMPH OF THE NEW ZEALAND EARWIG, *ANISOLABIS LITTOREA*

JOHN P. LEADER and JENNIFER J. BEDFORD

Zoology Department, University of Auckland, Auckland, New Zealand

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Abstract—The haemolymph of the maritime earwig, *Anisolabis littorea*, has been analysed for inorganic and organic components. The inorganic fraction has a high sodium ion concentration and osmotic pressure but a relatively lower chloride ion concentration. The organic fraction has a high amino acid content—the principal ones being proline, alanine, glutamic acid, and glutamine, and tyrosine. In contrast to the high aminoacidemia there is a very low carbohydrate content and no detectable trehalose in the haemolymph. The evolution of the composition of insect blood is discussed.

INTRODUCTION

THE ENDEMIC New Zealand earwig, *Anisolabis littorea* (White), is characteristically and commonly found under boulders around the high tidal mark on rocky shores. The relatively large size of this insect (3–4 cm), together with the wide range of environmental conditions which it is known to tolerate, makes it an interesting experimental animal for the examination of the rôle of osmotically active substances, in particular nitrogenous compounds, in the regulation of blood homeostasis. BEADLE and SHAW (1950), and SCHOFFENIELS (1960) have both postulated that the high aminoacidemia typical of insects may have a rôle to play in regulation of the blood osmotic pressure, particularly under conditions of chloride ion deplction.

In order to investigate the rôle of amino acids in the regulation of blood osmotic pressure, it became necessary to make a preliminary analysis of the normal blood composition. Apart from the work of CLARK (1958) and SUTCLIFFE (1963), no data on the blood of Dermaptera have been published. The aim of the present paper is to rectify this gap.

MATERIALS AND METHODS

Specimens of *Anisolabis littorea* were collected from the Whangaparoa Peninsula near Auckland. Only freshly collected animals were used for haemolymph analysis.

To collect haemolymph for analysis an earwig was held flat on a piece of Parafilm and 5 to 10 μl of haemolymph squeezed out from a previously cut

appendage. For some analyses pooled haemolymph was used. The haemolymph of earwigs clots slowly, and contains few blood cells, so that it was not found necessary to centrifuge the haemolymph before analysis.

Total osmotic pressure was determined by the microcryoscopic method of RAMSAY (1949), sodium and potassium ion concentrations, after suitable dilution on an EEL (Evans Electroselenium Ltd.) flame photometer, calcium and magnesium ion concentrations on an atomic absorption spectrophotometer (Southern Analytical 3000), and chloride ion concentration using the first potentiometric method of RAMSAY *et al.* (1955). Acid-soluble phosphate was determined by a modification (Bieleski, personal communication) of a standard colorimetric method (FISKE and SUBBAROW, 1925).

Total ninhydrin-positive substance was measured by the method of YEMM and COCKING (1955) using glutamic acid as a standard. Individual amino acids in deproteinized haemolymph were determined quantitatively by thin-layer electrophoresis and chromatography (BIELESKI and TURNER, 1966) followed by scanning with a Vitatron flying-spot densitometer (TLD 100) at 505 nm.

The principal proteins in the haemolymph were separated by acrylamide gel flat sheet electrophoresis using the method of REID and BIELESKI (1968), and staining with coomassie blue. After staining the gels were scanned on a Vitatron densitometer at 550 nm.

Total soluble carbohydrate in the haemolymph was measured using the anthrone method of YOUNG and RAISZ (1952). Total reducing substances were estimated by two methods: first, directly using the method of PARK and JOHNSON (1949), and second by determining the quantity of anthrone-positive substance remaining after hydrolysis of reducing sugars by heating 10 μl of blood with 0·2 ml 4 N sodium hydroxide at 100°C for 10 min. Glucose was estimated using glucose oxidase (RAABO and TERKILDSEN, 1960). The carbohydrate composition of the haemolymph was also investigated using both thinlayer and gas chromatography. For gas chromatography (Varian Aerograph Series 1700) an SE-52 column (nitrogen as the carrier gas) was used and the initial temperature of 215°C was increased after 8 min to 250°C at 20°C/min. Thin-layer chromatography was performed on silica gel-G buffered with 0·02 M sodium acetate using *n*-propanol–ethyl acetate–water (7 : 1 : 1) (TREVELYAN *et al.*, 1950) as a solvent, and sprayed with either fresh 10% sulphuric acid in water, or with an anthrone reagent (10% sulphuric acid (v/v) in a saturated ethanolic solution of anthrone) (WIMER *et al.*, 1970). All carbohydrate tests were also carried out on samples of haemolymph of the cockroach, *Periplaneta americana*, and these gave comparable results with those of TREHERNE (1960).

RESULTS

Total osmotic pressure and the inorganic constituents of the haemolymph

The total osmotic pressure, and the concentrations of the measured ions of the haemolymph are shown in Table 1. For an insect the total osmotic concentration

is high, and is largely the result of the contribution of sodium and chloride ions. There is, however, as in all other insects so far investigated, a large chloride ion deficit. When the other measured cations are taken into account, this amounts to about 90 mOsmole. It is unlikely that other inorganic ions such as sulphate or bicarbonate, could account for more than a small part of this difference.

TABLE 1—THE INORGANIC ION COMPOSITION OF THE HAEMOLYMPH OF THE EARWIG, *A. littorea*

Cations	Total osmotic pressure: 224·3 ± 20·6	Anions	
Sodium	193·0 ± 11·1	Chloride	116·9
Potassium	5·4 ± 1·2	Phosphate	3·0–4·0
Calcium	7·3 ± 1·0		
Magnesium	5·7 ± 0·9		
Total	224·4 mequiv.		127·4 mequiv.
Total cations and anions: 351·8 mOsmole			
Total osmotic pressure: 448·5 mOsmole			
Difference (anion deficit): 96·7 mOsmole			

Results are expressed in mM/l. haemolymph.

Organic constituents of the haemolymph

(a) *Amino nitrogen.* When measured as total ninhydrin-positive substances in terms of glutamic acid equivalents, the total concentration of amino acids was determined as 22·1 μg/μl haemolymph. When determined separately however by thin-layer chromatography and electrophoresis, the total concentration was found to be 36·1 μg/μl. Twenty amino acids were positively identified from the haemolymph of the earwig, and eight other ninhydrin-positive substances, probably peptides, were seen. The most abundant amino acids, making up more than 50 per cent of the total, were proline, glutamic acid and glutamine (which were not distinguished quantitatively), alanine, tyrosine, and taurine. Individual concentrations are shown in Table 2.

(b) *Soluble protein.* There were ten distinct bands in the protein pattern obtained from the haemolymph. Results from male and female earwigs gave similar patterns. A densitometer scan is shown in Fig. 1.

(c) *Carbohydrates.* The carbohydrate composition of the haemolymph is given in Table 3. Using gas chromatography it was found that glucose was present in moderate amounts (0·3 μg/μl haemolymph). This was also confirmed using glucose oxidase. In comparison with an equivalent amount of haemolymph from the cockroach *P. americana* in which trehalose is present in large amounts (TREHERNE, 1960) trehalose in *A. littorea* was undetectable (LEADER and BEDFORD, 1972).

Thin-layer chromatography confirmed the presence of glucose after spraying with sulphuric acid or anthrone reagent. Small amounts of other carbohydrates, as yet unidentified, were also found.

TABLE 2—THE CONCENTRATION OF AMINO ACIDS IN THE HAEMOLYMPH OF THE EARWIG, *A. littorea*

Amino acid	Concentration	Amino acid	Concentration
Alanine	3·8	Arginine	2·1
Cysteic acid	Present	Cystine	1·3
Glutamic acid and glutamine	6·7	Glycine	2·5
		Histidine	1·8
Isoleucine	2·9	Leucine	0·5
Lysine	1·6	Methionine	Present
Peptides	Present	Phenylalanine	Present
Proline	*ca.* 5·0	Serine	1·5
Taurine	6·3	Threonine	1·6
Tyrosine	2·7	Valine	0·5
Total: 36·1 μg/μl			

Results are expressed as μg/μl haemolymph.

TABLE 3—ESTIMATION OF THE CARBOHYDRATE CONTENT OF THE HAEMOLYMPH OF THE EARWIG, *A. littorea*

	Total carbohydrate	Total carbohydrate less reducing substances	Reducing substances	Glucose
Males	1·2 (3)	0·2 (1)	0·8 (1)	—
Females	1·8 ± 0·5 (10)	0·8 ± 0·3 (5)	0·7 ± 0·2 (7)	0·3 ± 0·1 (6)
All animals	1·7 ± 0·5 (13)	0·7 ± 0·4 (6)	0·7 ± 0·2 (8)	0·3 ± 0·1 (6)

* Figures in parentheses refer to the number of animals analysed in each case. Results are expressed as μg/μl haemolymph.

DISCUSSION

SUTCLIFFE (1963) recognized three stages in the evolution of the composition of insect blood. The primitive condition in which sodium and chloride are almost exclusively the main osmotic effectors evolves through an intermediate stage where organic molecules contribute more osmotically, to an advanced condition where all the inorganic ions are low in concentration and there is a much greater contribution to the osmotic pressure made by the organic components. The present results show that the Dermaptera, if *A. littorea* can be considered typical of this order,

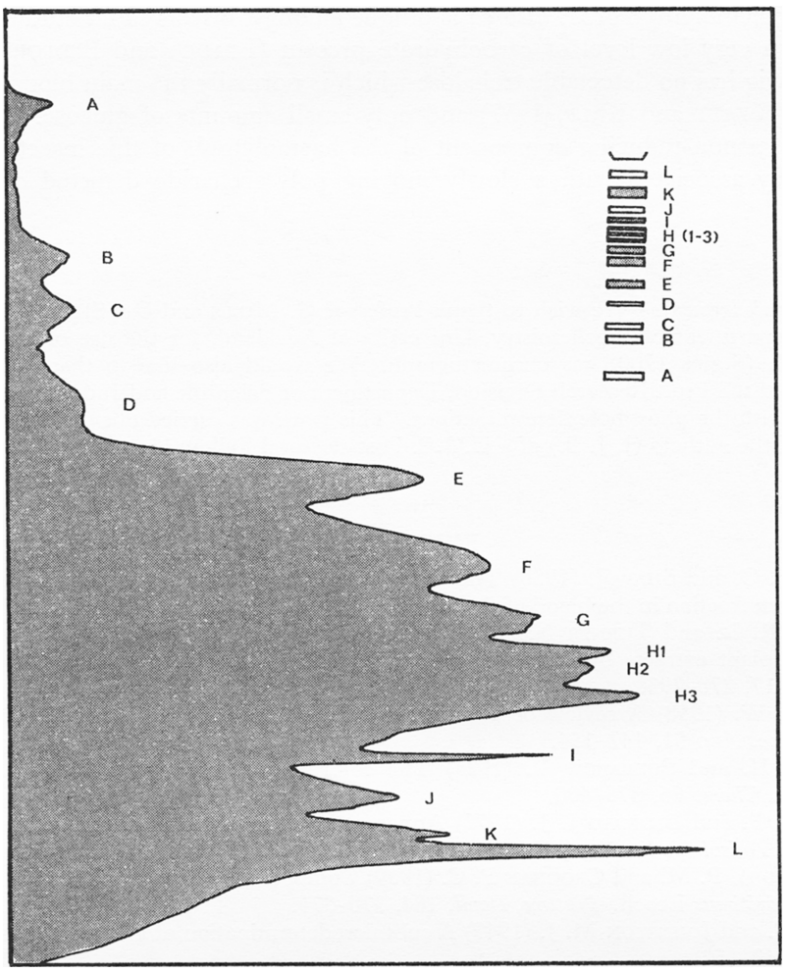

FIG. 1. Densitometer scan of the soluble proteins in the haemolymph of *Anisolabis littorea*. A–L are different bands corresponding to the pattern (inset).

agree with SUTCLIFFE's (1963) hypothesis. Most of the osmotic pressure of the haemolymph of *Anisolabis* is made up of sodium and chloride ions supplemented by a small organic component. The bulk of the amino acid contribution is made up of alanine, glutamic acid and glutamine, tyrosine, with a high concentration of proline.

The high osmotic pressure and sodium content of the haemolymph may be adaptively correlated with the maritime habitat of the earwig. *Anisolabis* is found supralittorally often in and amongst kelp and its omnivorous diet, which includes the isopod *Talorchestia*, and decaying seaweed, has a very high ionic content. The littoral thysanuran, *Petrobius maritimus* (LOCKWOOD and CROGHAN, 1959) has a similar high osmotic pressure and sodium ion concentration.

The haemolymph of *A. littorea* is unique amongst insects so far studied in that there is a very low level of carbohydrate present (LEADER and BEDFORD, 1972). The earwig has no detectable trehalose which is normally the main blood sugar of insects (WYATT and KALF, 1957) and only small amounts of glucose. There is, however, a non-reducing component of the haemolymph of this insect which is tentatively associated with a slowly moving polysaccharide detected chromatographically.

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