OSMOTIC AND IONIC REGULATION IN THE ISOPOD CRUSTACEAN *LIGIA OCEANICA*

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I. INTRODUCTION

Little is known of the physiology of isopods, and although much work has been done on the osmoregulation of other crustacean groups in both marine and fresh-water environments, the isopods have been rarely studied. The occurrence of related isopod species in marine, fresh-water and terrestrial habitats should provide interesting material for comparative physiological study. *Ligia oceanica* (L.) is the largest of the endemic species and so a suitable subject for experimental work. The recent development of a micro-freezing-point determination of osmotic pressure (Ramsay, 1949) and the micro-analytical techniques of Robertson & Webb (1939) make possible the analysis of the small quantities of blood obtainable from these animals.

This work was begun in order to find out if *L. oceanica* could control the concentration of salts and water in the blood in aquatic and terrestrial conditions. To this end the osmotic pressure of the blood was measured in animals living in varied conditions of salinity and oxygenation, and in moist and dry terrestrial conditions. The influence of moulting on the concentration of salts in the blood was also followed, but without any clear-cut results.

Previous measurements of the osmotic pressure of the blood in various isopod species have been made. Widmann (1935), using a cryoscopic method, gives a range of 2·2–2·5Δ° C. for normal *L. oceanica* collected from the shore; Bateman (1933) with vapour pressure determinations in the same species living on moist seaweed gives Δ2·29° C. (recalculated from the mean molar concentration given). Blood calcium was determined in the related *L. exotica* by Numanoi, who found 0·11 mg. calcium in 0·1 ml. blood (1934) and 0·97 mg. ± 0·06 in 1 ml. blood (1937). Of this total calcium, 75% was found to be dialysable and 25% bound to proteins.

II. METHODS

Experimental animals were kept in standard conditions of sand wet with sea water (from Plymouth, *c.* Δ1·98° C.) and were fed with seaweed (*Fucus* spp.). During experiments they were kept at room temperatures (18–20° C.). Osmotic changes could be followed in single animals as only 0·001 ml. or less of blood was required for the freezing-point determination; successive sampling had no measurable effect on the animal or on the freezing-point of the blood. Blood was drawn from the heart at the back of the thorax by a fine glass cannula. The small wound appeared

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to heal rapidly and there was little bleeding from the point of injury. For freezing-point measurements, animals were often used more than once; but when much larger blood samples were drawn for inorganic analysis, the animals were only used once, although they appeared to recover when replaced in suitable conditions.

Freezing-point measurements were all made in duplicate, the individual figures given in this paper being the means. The results are given as Δ° C. which may be converted into % NaCl concentrations by using the relationship Δ/NaCl (w/v) = 0·60 (Ramsay, 1949).

Inorganic analyses of the common ions were made by the following methods. All samples for cation determinations were first dried and then ashed with added sulphuric acid at 450° C. to remove organic matter. Sodium was precipitated and weighed as sodium zinc uranyl acetate; potassium was precipitated as potassium silver cobaltinitrite and then titrated with ceric sulphate; calcium was twice precipitated as oxalate and titrated with ceric sulphate. (These three methods were those of Robertson & Webb, 1939.) Magnesium was precipitated with hydroxyquinoline, the precipitate brominated and titrated iodometrically (Cruess-Callaghan 1935). Sulphate was precipitated with benzidine and the red colour (maximum absorption at 450μ) obtained by treatment with sodium β-naphthoquinone-4-sulphonate was compared with standard sulphate solutions in a Spekker photometer (Letonoff & Reinhold, 1936). Chloride was replaced by iodate which was determined iodometrically (Sendroy, 1937; Van Slyke & Hiller, 1947). Later analyses of chloride were made by Conway's microdiffusion technique (Conway, 1950, p. 184). This latter method gave results somewhat higher than the former. Total base was determined by converting bases to sulphate and measuring this gravimetrically as benzidine sulphate (Hald, 1933, 1934).

With the small quantities of blood available from single animals (0·1–0·5 ml.) the accuracy of the results obtained by these analytical methods was not so great as described in the original papers. Standard errors of the mean values were within 2·8 % for sodium, calcium and sulphate, 7·8 % for magnesium, 17 % for potassium, and 3 and 7 % for chloride depending on the method used. In view of the variations of Δ_{blood} from one individual to another, it seems likely that a large part of the observed variation in ion concentrations arises from a variation in the animals rather than from errors of analysis.

The volume of the blood samples was measured by weighing an equal quantity of distilled water in the same capillary pipette. Samples could not be weighed directly as the blood coagulated very rapidly. Concentrations of ions are expressed as mg./ml. blood and as m.equiv./l. The average water content was 92·65 % of the weight, about 954 mg./ml. (Specific gravity is assumed to be about 1·030.)

III. EXPERIMENTAL RESULTS

Osmotic pressure of the blood in standard conditions

Blood from animals in the conditions defined as standard had a freezing-point depression between Δ 1·983 and Δ 2·327° C. (mean Δ 2·15° ± 0·04 S.E., $n = 24$). In natural circumstances the variation within a population may be greater than this,

as the conditions in which the animals live are less rigorously defined. The mean value of Δ2·15° C. is greater than that normally observed in marine invertebrates, where the blood is usually isotonic with sea water (*c.* Δ1·98° C.). The other terrestrial members of the isopod group show much lower osmotic pressures (determined by the author by the same method):

	Mean Δ° C.	Range Δ° C.
Oniscus sp.	1·04 ($n=6$)	0·81–1·25
Armadillidium sp.	1·18 ($n=3$)	1·16–1·19
Porcellio sp.	1·30 ($n=3$)	1·25–1·37

The fresh-water genus *Asellus* has a blood salt concentration as low as that of other fresh-water invertebrates, with a mean freezing-point depression of Δ0·50° C. ($n=5$), range 0·41–0·62° C.

Moulting

The osmotic pressure of the blood of moulting individuals was determined in order to discover if it varied during the process of moulting. Three periods of moulting were defined: 'pre-moult', when calcium can be seen deposited in the anterior part of the integument, prior to the moult of the posterior half; 'mid-moult' between the moulting of the anterior and posterior halves; and 'post-moult' after the moulting of the anterior half. In the laboratory the time taken from the moult of the posterior to the moult of the anterior part of the integument was from 1 to 2 days. Non-moulting animals can be grouped into an 'inter-moult' stage of undetermined length.

Table 1. *Osmotic pressure of the blood of moulting stages*

Stage	Range, Δ° C.	No. of observations	Mean, Δ° C. ± S.E.
Intermoult	1·98–2·33	24	2·15 ± 0·04
Pre-moult	1·92–2·80	32	2·34 ± 0·04
Mid-moult	2·15–2·92	5	2·42 ± 0·13
Post-moult	2·25–2·87	11	2·55 ± 0·07

There is a general tendency for the osmotic pressure of the blood to rise during the moulting period. The observations which illustrate these changes are summarized in Table 1. '*t*' tests have indicated a significant rise between intermoult and pre-moult stages ($t=3·46$, 99% significance) and between pre-moult and post-moult stages ($t=2·47$, 98% significance). There is no indication of the sudden uptake of water after the moult which might be expected from Robertson's observations on *Carcinus* (1937). The blood of individuals kept for 2, 3 and 4 days after the moult still showed no subsequent drop in the osmotic pressure. (Observations in Table 2.)

Drying experiments

If animals are kept out of water in atmospheres of high humidity (90–100% R.H.) there is no change in the blood concentration. But if the relative humidity is lowered, there is a fairly rapid loss of water from the animal. Dry conditions for

Table 2. *Osmotic pressure of blood of individuals during moult*

Δ° C.$_{blood}$	Days of pre-moult				Day of mid-moult	Days of post-moult			
	1	2	3	4	5	6	7	8	9
Spec. 1	2·20	2·49	2·70	2·70	2·45	2·77	2·87	—	—
Spec. 2	2·26	2·42	2·74	2·80	2·92	2·86	2·85	—	—
Spec. 3	—	2·53	2·10	2·23	2·34	2·25	2·35	2·42	—
Spec. 4	—	—	—	2·14	2·26	2·37	2·38	2·50	2·46

Table 3. *Osmotic pressure of blood of individuals during drying: observations on successive days*

On wet sand	% R.H.	96	96	61	57	52	98
	Δ° C.$_{blood}$	2·12	2·10	2·20	2·47	2·98	2·46
On dry sand	% R.H.	86	93	87	79	76	98
	Δ° C.$_{blood}$	2·20	2·17	2·09	2·63	3·48	2·12

experiments were provided by suspending sulphuric acid solutions in closed breffits. The relative humidity was read from a paper hygrometer also suspended inside the jar. In one of these experiments, shown in Table 3, an individual was desiccated until Δ_{blood} was 3·48° C., without any permanent ill-effects since the blood returned to its normal range of concentration within 24 hr. in standard conditions. A second animal, inadvertently desiccated until Δ_{blood} was 3·75° C. (*c.* 6% NaCl), died shortly afterwards.

Immersion experiments

Ligia oceanica is not regarded as an aquatic marine animal, and various authors have reported difficulties in maintaining it in aquatic conditions. The present experiments have shown that this appears to be due primarily to asphyxiation of the animals; those which are kept in sufficiently well-aerated conditions can survive indefinitely. A suitable substratum is effective in reducing activity and so oxygen consumption.

Table 4. *Osmotic pressure of blood of individuals immersed in water*

Δ° C.$_{sea\ water}$	Δ° C.$_{blood}$							
	4 hr.	1 day	2 days	3 days	4 days	5 days	6 days	7 days
1·38	2·18	2·13	2·24	2·07	2·03	2·09	1·99	Dead
0·99	2·02	2·01	1·68	Dead	—	—	—	—
0·49	2·01	1·78	1·71	—	1·40	Dead	—	—

If animals are kept without aeration in 50 or 70% sea water the osmotic pressure of the blood falls appreciably (Table 4). Under these conditions the animals seldom survive for longer than a week, usually for less, but it is unlikely that the lowering of Δ_{blood} is the direct cause of death since still lower values can be sup-

ported for longer periods in aerated sea water (Table 5). In 25 % sea water without aeration the values of Δ_{blood} may be lower than any recorded in Table 5, and it is possible that in this case death is directly due to excessive dilution of the blood.

When the medium is aerated or oxygenated survival in 50–100 % sea water is indefinite, and in 25 % sea water may be as much as 17 days. The figures in Table 5

Table 5. *Osmotic pressure of blood after prolonged immersion. Medium aerated*

Days	Δ° C.$_{sea\ water}$	Δ° C.$_{blood}$
17	0·49	1·52, 1·59
30	0·99	1·99
30	1·38	2·09, 2·10
30	1·98	2·09, 2·10
30	2·17	2·35
47	2·47	2·46, 2·41
10	2·96	3·32
3	3·46	2·95

(Normal sea water Δ° C. = 1·98.)

refer to animals kept at 8° C., but even at 24° C. survival is indefinite in 50–100 % sea water. Experiments in which the animals were placed on filter-paper moistened with distilled water confirm the view that in the immersion experiments without aeration the cause of death is asphyxiation and not dilution of the blood. An animal taken from standard conditions was found to have a value of $\Delta_{blood} = 2{\cdot}12$° C.; after 20 hr. on filter-paper moistened with distilled water Δ_{blood} fell to 1·77° C. and after 48 hr. to 1·44° C.; but after return to standard conditions for 24 hr. Δ_{blood} had risen to 2·15° C. Similar changes, though in less degree, were observed when the filter-paper was moistened with 50 or 25 % sea water.

IV. IONIC REGULATION

The degree of ionic regulation in *Ligia* can be deduced from analyses of the common inorganic ions of the blood. Fluid from the maxillary glands was not obtained, so any possible part played by these organs in maintaining the blood concentration could not be determined. The figures for the mean values of the common ions are given in Table 6, together with those for sea water of Δ 1·98° C.

It is seen that the levels of sodium, potassium, calcium and chloride in the blood are higher than those in sea water, whereas the levels of magnesium and sulphate are lower. The total cations in the blood amount to 714 m.equiv./l. Of this total, 20 m.equiv. may be present as 'bound' calcium, since Numanoi (1934) estimates that in *L. exotica* a quarter of the calcium is bound to proteins. Total base determinations give a lower figure, 666 m.equiv./l. Total anions are 668 m.equiv./l., using the estimate of chloride obtained by Conway's method. To these might be added about 5 m.equiv. for phosphate (a single determination gave 0·073 mg. P/ml., most of which is present as HPO_4'' ion at blood pH), about 10 m.equiv. for proteinate, and about 5 m.equiv. for bicarbonate. Thus to balance 694 m.equiv. cations (714–720) there are at least an estimated 688 m.equiv. anions.

Table 6. *Concentrations of common inorganic ions in blood*

Ion	Blood			Sea water†		Concentration in blood as % of that in sea wate
	mg./ml. ± S.E.	N*	m.equiv./l.	mg./ml.	m.equiv./l.	
Sodium	13·47 ± 0·36	9	586	11·42	497	118
Potassium	0·54 ± 0·09	9	14	0·414	11	130
Calcium	1·45 ± 0·04	24	72	0·437	22	332
Magnesium	0·51 ± 0·04	14	42	1·378	113	37
Chloride‡	21·14 ± 0·66	14	596	20·58	581	103
Chloride§	23·36 ± 1·70	14	659	—	—	113
Sulphate	0·41 ± 0·01	13	9	2·874	60	14
Total anions	—	—	668	—	641	—
Total cations	—	—	714	—	643	—
Total base	—	3	666	—	643	—

* N is number of determinations.
† Δ 1·98° C.
‡ Sendroy's method.
§ Conway's method.

Total concentration of ions from the data of Table 6 is 1321 mg.-ions/l. or 1385 mg.-ions/kg. water, equivalent to about Δ 2·28° C. (using data on sea water from Sverdrup, Johnson & Fleming (1942, p. 67): Δ 1·872° = 1·1368 g.-ions/kg. water). But direct freezing-point data for *Ligia* blood (Table 1) give a mean value of Δ 2·15° C., equivalent to 1306 mg.-ions/kg. water. This difference in concentration may have resulted from different living conditions of different sets of animals, although all efforts were made to keep conditions as constant as possible. Or it may have arisen from a seasonal difference in the concentration of the blood, since Widmann (1935) gives a range, for males, from Δ 2·20° C. in August to 2·33° C. in November, and says that there is a fall in the concentration in April. Of the data given here, the osmotic pressure measurements were all made in April, May and June, while the inorganic analyses were made in October, November and December.

From the water content of *Ligia* blood the total solids can be calculated as 7·35 %, of which salts (from analysis) account for 4·04 %. A rough approximation of the protein content would thus be 3·3 % of the weight of the blood (33 mg./g.).

The relative proportions of cations bears some relationship to the physiological regulation of the animal (Florkin, 1949, p. 98) and perhaps to its activity (Robertson, 1949). The value of the ratio Na + K/Ca + Mg in the blood of many marine invertebrates does not differ appreciably from that in sea water, 3·8. For example, in the stone crab *Lithodes*, the blood has a value of 3·8, and it is very similar in composition to the sea water in which it lives; but other decapod crustaceans may show very different values, such as *Palinurus*, 9·2. *Ligia* occupies an intermediate position with a value of 5·3.

V. GENERAL DISCUSSION

Ligia oceanica is able to regulate the osmotic pressure of the blood when completely immersed in 75 or 100 % sea water provided that sufficient oxygen for the regulatory process is available. The blood is kept fairly constant in composition over this range,

but in lower or higher concentrations Δ_{blood} follows that of the outside medium. In terrestrial conditions the humidity of the environment is important in determining the blood concentration; water appears to be lost readily when the relative humidity of the air drops below 90%, if the animal has no access to water; there seems to be little control of water loss in dry air. Any water which is available is absorbed, and if only distilled water is present, the blood concentration is reduced. The general osmotic behaviour of *Ligia* does not lead us to suppose that it has any special physiological mechanisms which might enable it to embark successfully on a truly terrestrial existence. It is rather a marine animal which has developed a high degree of tolerance to internal osmotic changes, whether they are brought about by drying or by external osmotic changes. The evaporation of water in hot terrestrial conditions may, as Edney (1952) suggests, provide an adaptation for the cooling of the animal. It cannot be concluded from the evidence above that this species is intermediate either between the marine and the terrestrial members of the group or between the marine and the fresh-water members.

In the domain of ionic regulation it appears that *Ligia* can excrete or exclude magnesium and sulphate efficiently; calcium is accumulated to a notable extent, but about 25% of the total calcium may be bound to proteins; sodium, chloride and potassium are all higher in the blood than in sea water, especially potassium. The relative proportions of the inorganic cations indicate a highly developed inorganic regulation, comparable with that of some of the decapods.

Whether the urine is isotonic with the blood, as in marine and brackish-water crustaceans, or hypotonic to the blood, as in most fresh-water crustaceans, the problem of the source of salts remains. There are two possibilities: (1) that salt is absorbed from ingested food, and (2) that it is absorbed from the environment by some special region of the body. The first suggestion is a probable one, since the animal usually feeds on seaweed detritus, with a high salt content. But *Ligia* is reported 450 ft. above sea-level in the Outer Hebrides (Fraser Darling, 1947, p. 215); although seaweed detritus may be absent here, it presumably feeds on salt-encrusted vegetation. Absorption from the environment is possible either by swallowing the medium and absorbing it from the gut, or by selective absorption on some part of the integument. *Ligia* has a curious habit of dipping the uropods into water and draining the fluid over the pleopods. This behaviour may be merely a device for keeping the respiratory surfaces wet, but may also serve to regulate salt.

VI. SUMMARY

1. Osmotic pressure of the blood of *Ligia oceanica*, measured by the freezing-point depression, has a mean value of $\Delta 2{\cdot}15 \pm 0{\cdot}04°$ C. ($\equiv 3{\cdot}58\%$ NaCl on weight/volume basis).

2. Osmotic pressure of *Ligia* blood is much higher than that of other terrestrial isopods: *Oniscus* sp. $\Delta 1{\cdot}04°$ C.; *Armadillidium* sp. $\Delta 1{\cdot}18°$ C.; *Porcellio* sp. $\Delta 1{\cdot}30°$ C. or of the fresh-water *Asellus* sp. $\Delta 0{\cdot}50°$ C.

3. The osmotic pressure of the blood increases during the process of moulting, but no subsequent decrease is observed in the 4 days following.

4. Animals kept at low humidities lose water. They may be desiccated without permanent adverse effects until Δ_{blood} is 3·48° C. ($\equiv$5·8% NaCl). Recovery to a normal level takes about 24 hr. in moist conditions.

5. In well-aerated sea water between 50 and 100% concentration, animals survive without much alteration in Δ_{blood}. Above and below this range Δ_{blood} rises and falls.

6. In animals kept on filter-paper moistened with distilled water Δ_{blood} may fall to 1·44° C. ($\equiv$2·4% NaCl) without permanent adverse effects.

7. Analyses of inorganic ions in the blood show that sodium, potassium and chloride are all higher in concentration than in sea water; calcium is much more concentrated; and magnesium and sulphate much reduced.

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