THE OSMOTIC AND IONIC REGULATION OF *ARTEMIA SALINA* (L.)

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INTRODUCTION

The branchiopod Crustacea are mainly confined to fresh or only slightly brackish waters. A few species have evolved a tolerance to more saline media (Beadle, 1943*a*, *b*). Of these *Artemia salina* is the most successful, and has a wide-spread distribution in salt pools and salt lakes of high salinities.

In a previous paper (Croghan, 1958*a*), the survival of *Artemia* in various media is described. Prolonged survival only occurs in media in which certain sodium salts (principally NaCl) predominate. In sea-water media *Artemia* can survive actively over an extremely wide concentration range.

Early work suggested that *Artemia* was hypotonic to concentrated media. Abonyi (1915) found that in animals from a brine of about 10% NaCl, the chloride, expressed as NaCl, was equivalent to only 0·8% of the original wet weight. Later Martin & Wilbur (1921) found a very low ash weight in *Artemia*, and concluded that the concentration of the body fluids must be well below that of the medium. Other work has been mainly on samples of haemolymph obtained from the extensive haemocoel. Medwedewa (1927) made a few determinations of the osmotic pressure, and concluded that the haemolymph was markedly hypotonic to the medium. Warren, Kuenen & Baas Becking (1938) measured the sodium and chloride concentrations in the haemolymph. Their paper is full of errors but they confirm that the haemolymph is hypotonic to the medium, and that NaCl is the most important osmotic constituent of the haemolymph. Kuenen (1939) continued this work, and extended somewhat the range of external concentration over which hypotonic regulation was known to occur. More recently Plattner (1955) measured the haemolymph osmotic pressure of *Artemia* from media containing from 2 to 28·5% total salts, and found a very marked hypotonicity even in the most concentrated media. He also found that in animals transferred to distilled water there was a fairly rapid fall in the haemolymph osmotic pressure. Another aspect was studied by Ussing (in Krogh, 1939), who found a rather slow exchange of D_2O. As the animal is therefore to some extent permeable, an osmo-regulatory mechanism must be present.

It is clear that *Artemia* has evolved a mechanism that maintains the haemolymph hypotonic in highly saline media. In the present paper the osmotic pressure and chemical composition of the haemolymph and the chemical composition of the whole animal in relation to the composition of the medium is described. Evidence concerning permeability and active transport will also be presented.

MATERIAL AND METHODS

Adult *Artemia* (about 10–12 mm. in length) from a sea-water culture described previously (Croghan, 1958*a*) were used. Experiments were done within the temperature range 18–24° C.

The animals have been acclimatized to a very wide range of external salinities. Low salinities were produced by diluting sea water, and higher salinities by boiling down sea water and re-aerating. Over a wide range the animals can be transferred from one medium to another with a low mortality incidence. In the more extreme ranges the mortality is greater, but is lessened if the animals are acclimatized over a period of days by gradually diluting or increasing the concentration of the medium. In the case of the most concentrated media (greater than 600% sea water) acclimatization was obtained by allowing evaporation to proceed until, after several weeks, NaCl began to crystallize.

Haemolymph samples

The animal was removed from its medium with a rubber teat pipette, rinsed rapidly in distilled water, dried on filter paper and cigarette paper, transferred to a slide and covered with liquid paraffin. A collecting pipette was made by drawing out a piece of 2 mm. diameter glass tubing to a sharp tip. The pipette was attached to a length of thin rubber tubing, the other end of which was held in the mouth. Liquid paraffin was sucked into the pipette. Working under a low-power binocular microscope, the body wall was punctured with a needle over the dorsal vessel, and the cherry-red haemolymph was sucked into the pipette. From a large adult 10–12 mm. long, and weighing about 8 mg., about 1–2 μl. of haemolymph could easily be obtained. The animals did not survive the sampling procedure. The sample was stored under liquid paraffin in a lacquered watch-glass. When it was not possible to analyse the samples immediately they were stored in a refrigerator or deep-freeze. Fortunately, the blood does not coagulate.

Whole animal samples

For each sample thirty to thirty-five animals were pipetted from their medium, rinsed quickly in distilled water, and dried on filter paper. Final traces of adherent water were removed by sprinkling the animals on the filter paper with a few drops of acetone, and finally drying them quickly with an air jet. The animals were transferred to a silica crucible and the wet weight was determined. The water content was found by difference after heating at 105° C. for 6–8 hr. The samples were then ashed at 550° C. for 30 min. For chloride determinations small quantities of either $KHCO_3$ or $NaHCO_3$ were added before ashing to reduce possible loss of this ion. 1 ml. of 20% sulphuric acid was added to the ash, and the extract was used for chemical analyses. The methods used were the same as for haemolymph except that larger micropipettes were used. The concentrations of sulphate and phosphate in the final solutions were well below the interference levels of the flame-photometer.

Osmotic pressure

This was determined using the micro-cryoscopic method of Ramsay & Brown (1955). Results were repeatable to within 0·01° C. Samples were usually done in duplicate or triplicate. The osmotic pressure was expressed empirically in terms of that concentration of NaCl solution that would give the same depression. Over the range 0–5 %NaCl, the freezing-point depression, Δ, was a linear function of concentration with a conversion factor of % NaCl $= \Delta/0{\cdot}60$.

Checks showed that there was no appreciable change in freezing-point of haemolymph stored under liquid paraffin and left at room temperature overnight. But whenever it was necessary to store a sample this was done at low temperature as an added precaution.

Chloride concentration

The first method of Ramsay, Brown & Croghan (1955) was used. For haemolymph samples micropipettes of 0·5–1 μl. proved most convenient. The pipettes were calibrated using standard NaCl solutions. With both standards and biological fluids the standard deviation of a series of titrations was not greater than 1 %. As far as possible titrations were done in duplicate or triplicate.

Sodium concentration

This was determined using an EEL flame-photometer. With haemolymph it was possible to do a determination on about 0·5–1 μl. A sample was transferred with a micropipette into 2 ml. of distilled water in a small polythene tube, and mixed by a gentle stream of air-bubbles. Standards were prepared in a similar way. The standard deviation of a series of similar samples giving nearly full-scale deflexion at maximum sensitivity was about 1–3 %. Whenever possible samples were done in duplicate or triplicate.

Potassium concentration

This also was determined with the EEL flame-photometer. The technique was the same as for sodium determinations. Rather large volumes of haemolymph had to be collected for a single determination (10–15 μl.), and this meant pooling the haemolymph from several individuals. It was not always possible to run a duplicate unknown, and thus the accuracy is likely to be somewhat less than in the case of sodium.

Magnesium concentration

This was determined by a modification of the method of Orange & Rhein (1951). A haemolymph sample (20–40 μl.) was deproteinized by adding an equal volume of 10 % trichloracetic acid and centrifuging. The supernatant was pipetted off, and added to 1 ml. of a very dilute Titan yellow solution in a polythene tube. 1 ml. N-NaOH solution was then added. The tube was stoppered and well shaken, and the extinction relative to a blank was read off on a Spekker absorptiometer, using 2 cm. light path microcells, and green filters. A series of standards was also prepared. With the limited volumes of haemolymph available the method was being pushed to the limit of its sensitivity. High accuracy could not therefore be expected.

Phosphate content

Phosphate was not detectable in the available volumes of haemolymph, but estimations were made on the phosphate in the ash of whole animals. A modification of Delory's method (1949) was used. A mixture of 1 ml. of the aminonaphthol-sulphonic acid reagent and 2 ml. of the acid ammonium molybdate reagent was diluted to 20 ml. 1 ml. of this mixture was then placed in each of a series of tubes, and a measured volume of unknown or of one of a series of standards was added to each with a micropipette. The extinction was determined on a Spekker using 2 cm. light path microcells and red filters. The standard deviation of a series of similar standards was less than 1%.

RESULTS

Composition of the haemolymph

Animals were left in a new sea-water medium for at least 2 days, and in many cases considerably longer before the haemolymph was sampled. The samples were either from single animals, or were pooled samples from up to twenty animals or more.

The haemolymph osmotic pressure was determined. A sample of the medium was also measured in comparison. The more concentrated media had to be diluted considerably to bring them within the range of the thermometer. The results were then multiplied by the dilution factor. The highest values for the medium are apparently above the maximum solubility of NaCl. This presumably means that a crystallizing sea-water brine has a higher osmotic pressure than a saturated NaCl solution. Thus, an apparent anomaly appears when the osmotic pressure is expressed in terms of NaCl.

The results are plotted out on two graphs (Figs. 1, 2) to cover the very wide external salinity range. Also included in these graphs for convenience are data on the osmotic pressure of the gut fluids. This will be discussed in a subsequent paper (Croghan, 1958*c*).

The results extend the range and precision of the observations by earlier workers, and demonstrate clearly the relative constancy of the haemolymph concentration and its independence from that of the medium. Over a range of medium concentration increasing by a factor of about 100, the haemolymph concentration increases by a factor of only about 6. Although this increase is relatively small, it still indicates that a considerable toleration by the tissues to changes in total haemolymph concentration is required.

In the more concentrated media the hypotonicity of the haemolymph is very marked. In media more dilute than 25% sea water, *Artemia* is hypertonic to its medium. It is behaving like a brackish-water organism. But the most dilute sea-water medium to which it has been possible to adapt *Artemia*, even over a period of several weeks, is still only as dilute as 0·26% NaCl. In fresh water, *Artemia* dies in about 24 hr.

The osmotic pressure of the haemolymph of *Artemia* from dilute media is not much different from that of the fresh-water species *Chirocephalus diaphanus*. In this animal Panikkar (1941*a*) found a range of haemolymph osmotic pressure of 0·44–0·50% NaCl. In the present work haemolymph samples from three large specimens were tested separately. The mean osmotic pressure was found to be 0·43% NaCl.

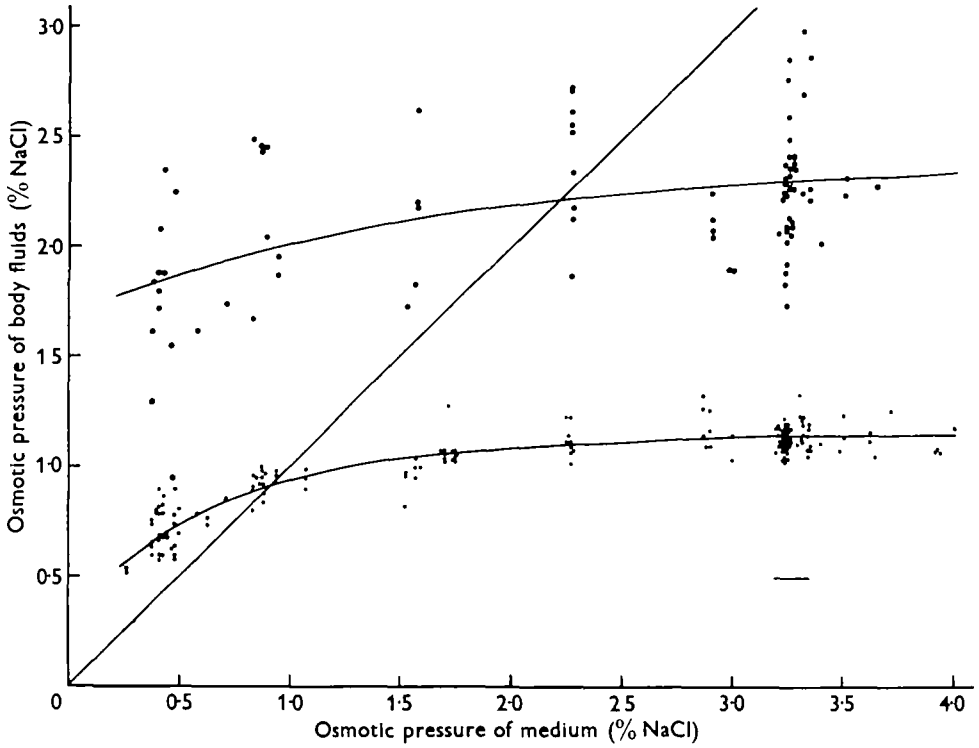

Fig. 1. The relation between the osmotic pressure of the body fluids and the medium in the more dilute media. Haemolymph osmotic pressure, ●; gut fluid osmotic pressure, ○. The short horizontal line represents the range of values of ordinary sea water. The diagonal line through the origin represents isotonicity.

In view of the marked hypotonicity of *Artemia* adults in concentrated media, it was considered of interest to examine nauplii. Haemolymph has been obtained from a few 2nd instar nauplii (criteria of Heath, 1924). A nauplius (0·5–0·8 mm. long) was pipetted from the sea-water medium, in which it had hatched, onto a slide, the adherent sea water was carefully removed with cigarette paper fragments, and the nauplius was covered by a drop of liquid paraffin. The neck organ was punctured with the fine silica capillary. A haemolymph sample was drawn up, and the osmotic pressure determined. The sea-water medium was found to be 3·32% NaCl, and the mean result for the haemolymph concentration of five nauplii was $1{\cdot}30 \pm 0{\cdot}17$% NaCl. These results clearly indicate that the nauplii are hypotonic to sea water, and have a haemolymph concentration fairly similar to that of the adult.

Chemical analyses have been carried out on many of the haemolymph samples whose osmotic pressures are recorded in Figs. 1 and 2. In many cases several ions were determined in the same pooled sample, and this was particularly so in the case of sodium and chloride. Information has been obtained for animals acclimatized to a range of external salinities varying from 10 to 600% sea water. For convenience and clarity these results are presented as a function of haemolymph osmotic pressure (Fig. 3).

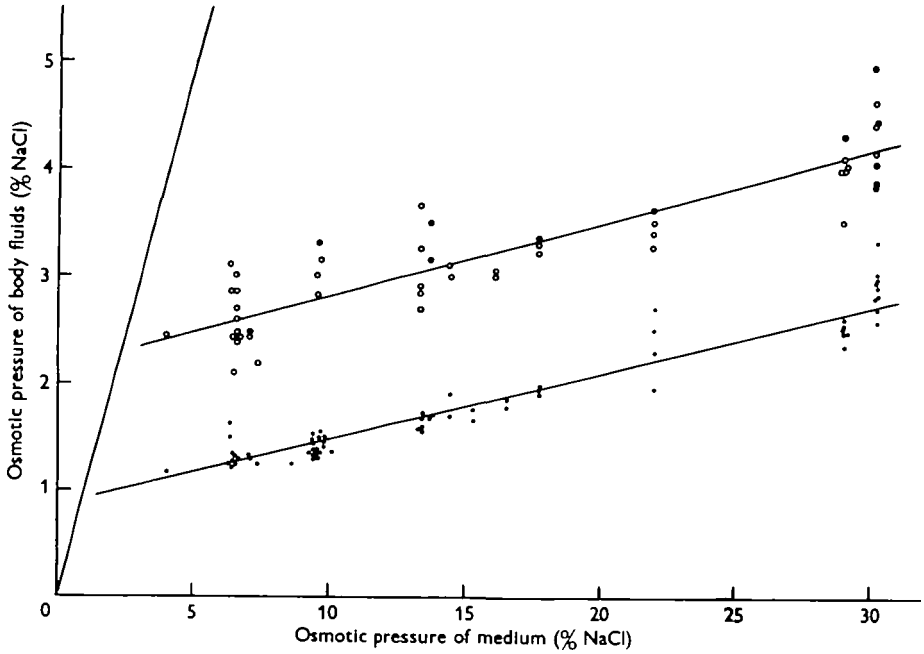

Fig. 2. The relation between the osmotic pressure of the body fluids and the medium in the more concentrated media. Haemolymph osmotic pressure, ●; gut fluid osmotic pressure, O. The diagonal line through the origin represents isotonicity.

It is clear that ionized sodium salts can account for about 90% of the total haemolymph osmotic pressure. The chloride equivalence is some 10% lower. These two ions account for almost all the observed osmotic pressure (as is probably general in Crustacea), and the ratio of their concentrations does not change appreciably over the range of haemolymph osmotic pressure studied. Potassium and magnesium ions are very much less important osmotic constituents.

Chemical analyses were also carried out on some of the media from which the animals were taken. As these media covered a wide range of osmotic pressure, the data have been adjusted to the osmotic pressure of ordinary sea water. The mean results are as follows: osmotic pressure of sea water, $\Delta = 1{\cdot}95°$ C. (= 3·25% NaCl); chloride, 565 mM./l.; sodium, 485 mM./l.; potassium, 11 mM./l.; magnesium, 55 mM./l.

The haemolymph ionic ratios are very different from those of the medium. The Na:Cl ratio of the sea-water brine is 0·86, whereas in the haemolymph the ratio is always greater than one. The haemolymph magnesium concentration is extremely low, and is well below that of the sea-water brines (Fig. 4). The potassium concentration is held relatively constant in the haemolymph, and can be very different

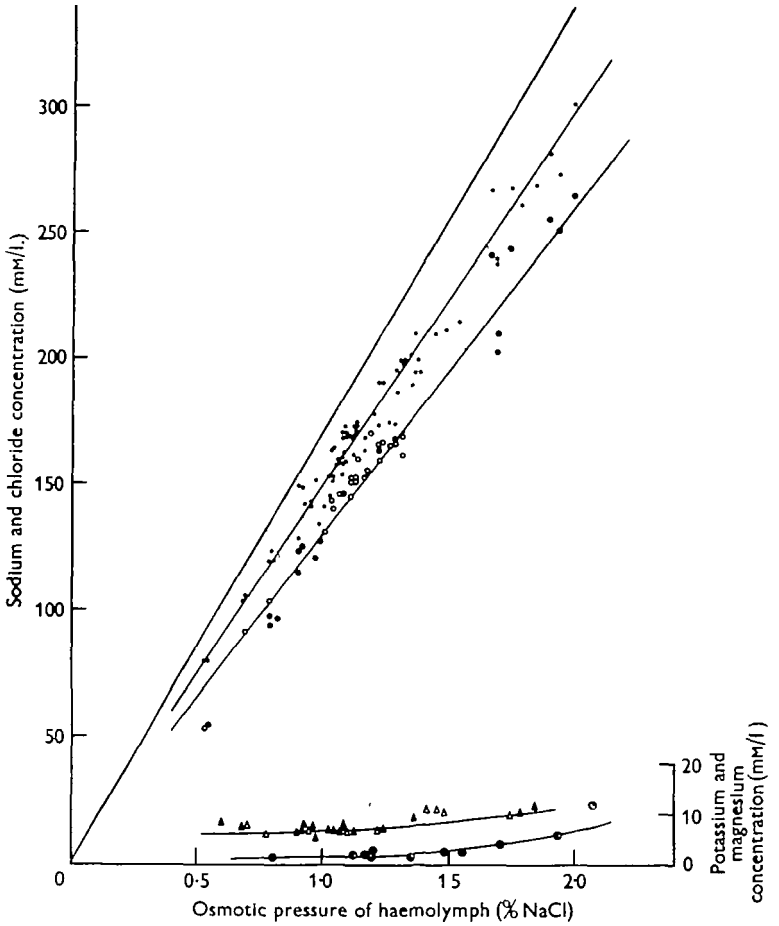

Fig. 3. The chemical composition of the haemolymph. Sodium, ●; chloride, ○; potassium, △; magnesium, ⊙. The diagonal line through the origin represents the values expected if all the haemolymph osmotic pressure was accounted for by sodium and chloride ions.

from that of the medium (Figs. 3, 4). The ratio K:Na in the haemolymph compared with that in the medium ($K_t \times Na_m/Na_t \times K_m$) is also plotted in Fig. 4. This enrichment factor is always greater than unity, even when the actual potassium concentration in the medium is well above that in the haemolymph. This relative elevation of the haemolymph K:Na ratio is a further example of one of the most widespread features of animal blood.

Shortage of material prevented a detailed comparative study of the haemolymph of fresh-water branchiopods. However, a few measurements were made on *Chirocephalus diaphanus* from fresh water. Haemolymph samples from the three large specimens previously mentioned were analysed separately. The mean values are: osmotic pressure, 0·43% NaCl (73·5 mM./l.); sodium, 62 mM./l.; chloride, 51 mM./l. The Na: Cl ratio, and the contribution of these two ions to the total osmotic pressure in *Artemia* are very similar to these *Chirocephalus* values.

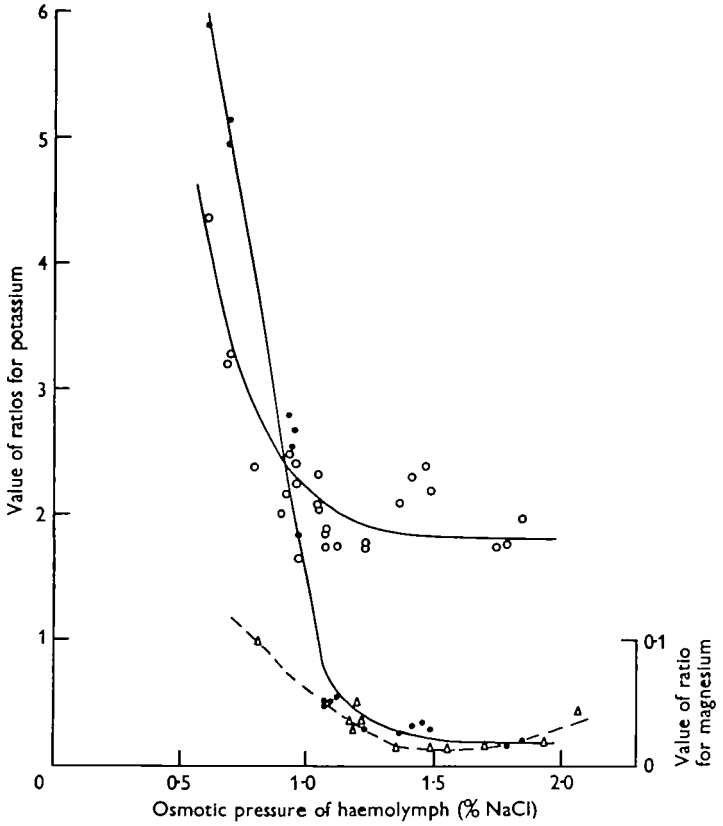

Fig. 4. The values of some ionic ratios. K_i/K_m, ●; $K_i \times Na_m/Na_i \times K_m$, ○; Mg_i/Mg_m, △. (i is haemolymph and m is medium.)

The curves relating the osmotic pressure of *Artemia* haemolymph to that of the medium (Figs. 1, 2) have a definite slope, and these changes in haemolymph osmotic pressure are due to changes in the NaCl concentration in the haemolymph (Fig. 3). This is evidence of permeability. But, as the haemolymph composition is still very different from the medium, the haemolymph steady state must be maintained by active mechanisms that balance the passive diffusion movements of water and ions. This becomes clearer if we analyse more closely some of the data included in Figs. 1–3. In the experiment recorded in Table 1, the osmotic pressure and sodium

concentration were both determined on the haemolymph from single large animals. Group 1 were animals from a culture that had been kept in 50% sea water for 4 days. The rest of the animals were then transferred to 300% sea water. After 2 days haemolymph samples were taken (Group 2). The remaining animals were then divided into two groups. Group 3 was left in the 300% sea water for a further 2 days before sampling, and group 4 was transferred back to a 50% sea water, and left for 3 days before sampling. During the experiment the mortality due to transferring the animals to media of different concentration was extremely low, and thus selection

Table 1. *The osmotic pressure and sodium concentration of the haemolymph of individual animals*

Group	Medium		Haemolymph	
	Osmotic pressure (% NaCl)	Na mM./l.	Osmotic pressure (% NaCl)	Na mM./l.
1	1·75	246	1·05 ±0·02 (6)	156± 6 (6)
2	9·37	1380	1·39 ±0·06 (7)	205± 8 (7)
3	9·37	1380	1·36 ±0·06 (4)	197±10 (4)
4	1·70	242	1·055±0·02 (5)	156± 3 (5)

The mean values and the standard deviations of each group of individual animals are given. The figures in brackets are the number of individuals studied.

Table 2. *The effect of distilled water on the composition of the haemolymph*

Medium	Haemolymph			
	Sample group	Osmotic pressure (% NaCl)	Na mM./l.	Cl mM./l.
Original sea-water medium (osmotic pressure 3·63% NaCl)	1	1·125	172	153·5
	2	1·16	169	153·5
Distilled water (frequently changed) 13 hr. exposure	3	0·59	79·5	71
	4	0·615	72	60

could not have influenced the results. On transferring animals to a higher concentration the osmotic pressure rises slightly but definitely, due to an increase in sodium salts, to a new steady state. This new level is reached and stabilized within 2 days. On transferring some animals back to the dilute medium, the haemolymph osmotic pressure and sodium concentration fall to the original level again, although the osmotic pressure and sodium concentration of this medium is still above that of the haemolymph. This experiment clearly demonstrates permeability, and that the animal can actively decrease the haemolymph osmotic pressure whilst still in a hypertonic medium.

In glass-distilled water, *Artemia* dies in about 24 hr. In Table 2 some haemolymph analyses are given. Each group refers to a sample pooled from several individuals.

It seems clear that death in distilled water is due to a rapid loss of NaCl and/or gain of water. This demonstrates a considerable degree of permeability, and indicates that in dilute media, to which *Artemia* is hypertonic, active mechanisms for taking up NaCl and excreting excess water must be present.

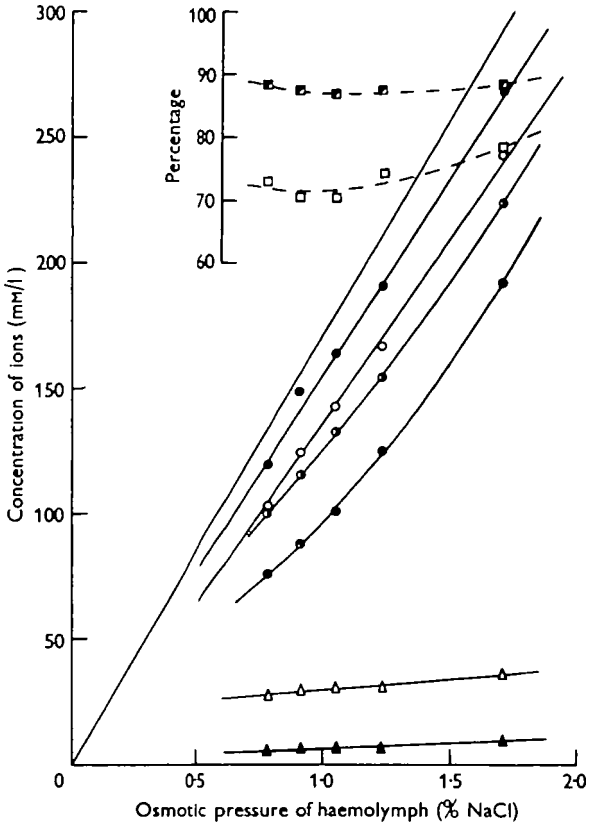

Fig. 5. The chemical composition of the whole animal in comparison with the haemolymph. Sodium concentration in haemolymph, ●; in T.B.W., ◑. Chloride concentration in haemolymph, O; in T.B.W., ⊙. Potassium concentration in haemolymph, ▲; in T.B.W., △. T.B.W. as percentage of original weight, ▩. Chloride space as percentage of T.B.W., □. (T.B.W is total body water.)

Composition of the whole animal

Animals were acclimatized for several days in clean filtered media varying from 15–500% sea water. Two series of samples were prepared. One ashed with $KHCO_3$ was used for chloride and sodium estimations, and the other ashed with $NaHCO_3$ was used for chloride and potassium estimations. Results are expressed as mM. per litre of total body water (T.B.W.). Comparative analyses were also made on the haemolymph of groups of animals removed from the media at the same time as the samples for ashing.

The results are plotted in Fig. 5. The percentage of water in the animal remains

fairly constant. The concentrations of sodium and chloride in the T.B.W. rise closely parallel to and not much below the haemolymph values. If the chloride is assumed to be entirely extracellular, the chloride space (Cl conc. in T.B.W./Cl conc. in haemolymph) gives the haemolymph volume (extracellular space). This chloride space is plotted in Fig. 5. It is relatively constant and very high. The ease with which large amounts of haemolymph can be obtained also indicates a large haemolymph space. The animal gives the impression of being an elastic sac, in which shape and mechanical rigidity are maintained by haemolymph pressure, the actual tissues occupying a relatively small volume. This is confirmed by the fact that, although the potassium concentration in the T.B.W. is higher than in the haemolymph, as one would expect for a mainly intracellular ion, its concentration in the T.B.W. is still quite low.

It is of interest to determine how the changes in external salinity affect the total water and ion content of the animals, as distinct from concentration. For this purpose the phosphate content of the acid ash extracts, used to obtain the data for Fig. 5, was also determined. As there is very little phosphate in the haemolymph or the medium and large amounts in the ash extracts, it is clear that the phosphate is derived from intracellular phosphorus compounds. It is reasonable, therefore, to consider that the phosphate content of the ash is unlikely to have been affected by the various external salinities used in these experiments. Changes in the ratios of total water content and the total amount of various ions present to phosphate content therefore indicate net movements of these substances into or out of the animal. The mean values for the animals from the most dilute medium used (osmotic pressure = 0·48% NaCl) have been put equal to 100, and the ratios for the animals from the other more concentrated media have been expressed as percentages of this. The results are presented in Fig. 6. It is clear that the changes in haemolymph osmotic pressure in different media are accounted for more by changes in the NaCl content of the animal than by changes in water content, and this is direct evidence that the animal can actively excrete NaCl against the concentration gradient. The potassium of the animal, as would be expected for a mainly intracellular ion, only rises slightly in the increased external salinities.

Localization of permeability

The preceding sections have indicated that *Artemia* is permeable. Further information was obtained by placing animals in a mixture of sea water and glycol. Animals from a sea-water culture (= 3·24% NaCl) were placed in sea water plus 15% (v/v) glycol and a little phenol red. In this medium they survived well for the duration of the experiment. Some of the animals were previously ligatured tightly at the neck and anus with fine fibres teased out of strands of bolting silk. Such ligatured animals have survived with active swimming movements for a day or more. The phenol red showed that the unligatured animals were actively swallowing the medium, whereas the ligatured ones could not do so. Haemolymph samples were easily obtained from both unligatured and ligatured animals, and there was no sign of appreciable dehydration in either.

The results are graphed in Fig. 7. Each point represents a single animal. The osmotic pressure rises far more rapidly in the unligatured animals than in the ligatured animals. The results demonstrate a considerable degree of permeability. The ligaturing experiment clearly demonstrates that most of this permeability is

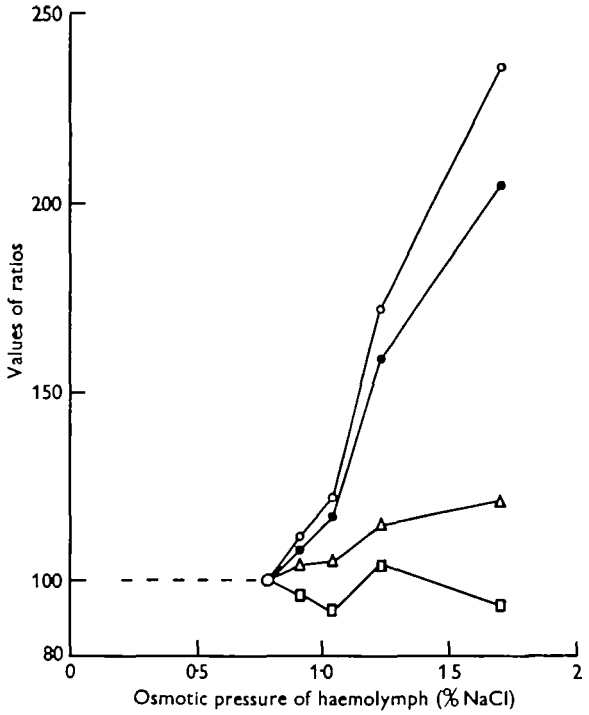

Fig. 6. The chemical composition of the whole animal relative to phosphate content. Sodium/phosphate, ●; chloride/phosphate, O; potassium/phosphate, △; water/phosphate, □.

across the gut epithelium, the external surface being much less permeable. After 23 hr. exposure, the haemolymph chloride concentration was also determined in five ligatured animals, and in the five unligatured animals that had the highest haemolymph osmotic pressure. The results are given in Table 3. It is clear that the rise

Table 3. *The composition of the haemolymph of some animals after 23 hr. in the medium containing sea water and glycol*

Unligatured animals		Ligatured animals	
Osmotic pressure (% NaCl)	Cl mM./l.	Osmotic pressure (% NaCl)	Cl mM./l.
3·85	149	1·35	137
3·67	144	1·44	123
3·89	142	1·72	116
3·00	152	1·35	137
3·52	142	1·53	104

in osmotic pressure in the unligatured animals is not due to osmotic dehydration and a resultant rise in chloride concentration, but must be due to the entry of the relatively large glycol molecules across the gut epithelium.

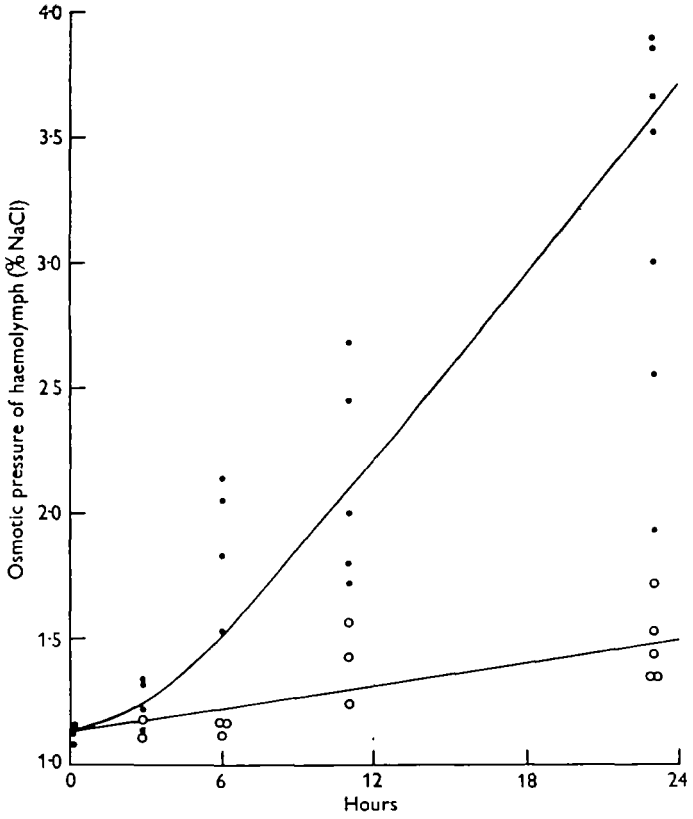

Fig. 7. The effect of a medium containing sea water and glycol on the osmotic pressure of the haemolymph. Normal unligatured animals, ●; ligatured animals, ○.

DISCUSSION

It has been shown that the osmotic pressure and ionic composition of *Artemia* haemolymph can be very different from the medium. Even in concentrated media, the haemolymph osmotic pressure is still less than that of a typical marine invertebrate in sea water, and is more like that of a fresh-water animal in fresh water. Further, the ionic composition of the haemolymph has characters more like those of a fresh-water animal, e.g. a high Na:Cl ratio, and an extremely low magnesium concentration. All this is physiological confirmation of a fresh-water ancestry, and indicates that the evolution of *Artemia* has been a process of superimposing upon a basically fresh-water type of physiology a method of stabilizing the haemolymph composition at a level which the cells and tissues can tolerate. This has enabled the animal to colonize extremely saline media that are ecologically open habitats.

Artemia, contrary to the statement of Beadle (1943*a*), also possesses the ability to maintain a hypertonic haemolymph in dilute media, but this ability is limited and appears more a 'vestigal character', and the animals cannot survive long in fresh water or distilled water.

A number of other animals have been shown to be markedly hypotonic to saline media. These include *Aedes detritus* larvae (Beadle, 1939), the marine teleosts (Smith, 1932), and certain palaemonids (Panikkar, 1941*b*). The ability of these animals to live in concentrated media is appreciably less well developed than in *Artemia*, although *Aedes detritus* can live in brines up to at least 10% NaCl (Beadle, 1939). All these hypotonic forms seem to be of fresh-water origin, and to have had a similar evolutionary history to *Artemia*.

The experiments have shown that *Artemia* is appreciably permeable. In hypertonic media *Artemia* would tend constantly both to gain NaCl and to lose water, and, it is clear that in these media the haemolymph steady state must be maintained by well-developed active transport mechanisms both for excreting NaCl and for taking up water. The demonstration that the animal can decrease the osmotic pressure and NaCl concentration of the haemolymph and the NaCl content of the whole animal whilst still in a hypertonic medium is direct evidence for such mechanisms.

The low permeability of the external surface of *Artemia* may be regarded as an adaptation hindering passive diffusion of NaCl and water. Most of the permeability of the animal is across the gut epithelium, and it seems clear that in hypertonic media it is across this epithelium that there will be the greatest tendency for NaCl to enter and for water to leave the haemolymph. A considerable permeability across the gut epithelium would be expected as various products of digestion have to be taken up and as the cuticle which covers the external surface is in the gut only represented by a thin peritrophic membrane. In *Aedes detritus* larvae Beadle (1939) found that the permeability was similarly restricted.

The nature of the active transport mechanisms in *Artemia* in comparison with those in other organisms showing hypotonic regulation is of considerable interest and will be discussed in subsequent papers (Croghan, 1958*b*, *c*).

SUMMARY

1. It has been possible to adapt *Artemia* to sea-water media varying from 0·26% NaCl to crystallizing brine. In fresh water or distilled water survival is relatively short.

2. The osmotic pressure of the haemolymph is relatively independent of the medium and increases only slightly as the medium is made more concentrated. In the more concentrated media the haemolymph is very markedly hypotonic. In media more dilute than 25% sea water the haemolymph is hypertonic. In distilled water there is a rapid fall of haemolymph concentration. The haemolymph of nauplii from sea water is hypotonic.

3. The sodium, potassium, magnesium, and chloride concentrations of the haemolymph have been determined. The bulk of the haemolymph osmotic pressure

is accounted for by sodium and chloride ions. The ionic ratios of the haemolymph are relatively constant, and very different from those of the medium.

4. The concentrations of ions in the whole animal have been studied. The chloride space is extremely high. Such changes in haemolymph osmotic pressure that do occur as the medium concentration is varied are due more to net movements of NaCl into or out of the body than to water movements.

5. Evidence is collected to show that an appreciable degree of permeability exists. Most of this permeability is localized to the gut epithelium, the external surface being much less permeable.

6. It is clear that *Artemia* must possess mechanisms that can actively excrete NaCl and take up water in hypertonic media. It has been demonstrated that *Artemia* can lower the haemolymph osmotic pressure by excreting NaCl from the haemolymph against the concentration gradient.

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