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# IONIC REGULATION AND MODE OF ADJUSTMENT TO REDUCED SALINITY OF THE STARFISH *ASTERIAS RUBENS* L.

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(Text-figs. 1–6)

In their usual marine habitat echinoderms are not normally subjected to great changes in either the osmotic pressure or ionic composition of the environment. Lacking, as they do, any differentiated excretory organ, it is not surprising to find that the body fluids approximate in composition to sea water. Many workers have determined the osmotic pressure or chloride content of the perivisceral fluid under environmental conditions and some have included data from animals subjected to reduced salinity (Bottazzi, 1897; Quinton, 1899; Fredericq, 1901; Henri & Lalou, 1903*a*, *b*, *c*, 1904; Garrey, 1904; Bottazzi, 1906, 1908; Dakin, 1908; Myers, 1920; Duval, 1924, 1926; Irving, 1926; Schlieper, 1930; Vetokhin, 1931; Maloeuf, 1938; Binyon, 1961). More detailed chemical analyses have been performed by Griffiths, 1892; Koizumi, 1932; Bialaszewicz, 1933; Robertson, 1939; Cole, 1940; Robertson, 1953; and some have extended their work to include an investigation of the fluid from the water vascular system (Bethe & Berger, 1931*a*; Parker & Cole, 1940; Robertson, 1949). Determinations on the gross chemical composition have been recorded by Galtsoff & Loosanoff, 1939; Vinogradov, 1953; and of certain organic constituents by Giordano & Harper, 1950, and Schlieper, 1957.

Apart from isotonicity with the medium, even if this should become diluted, the only point of major interest to emerge from these results is the fact that certain echinoderms show a slightly enhanced potassium concentration in the water vascular (ambulacral) fluid. In view of the existence of this very limited power of ionic regulation, it was decided to make as full an investigation as possible into the chemical composition and regulatory powers of *Asterias rubens* from the North Sea.

## MATERIAL AND METHODS

The animals were collected at Whitstable, which is situated at the mouth of the Thames estuary on the north Kent coast. After dabbing with a cloth to prevent contamination with sea water, the perivisceral fluid was obtained by snipping off the tips of one or more arms and the fluid allowed to drain into

a centrifuge cone. The collection of sufficient ambulacral fluid without contamination with perivisceral fluid presented a more difficult task. One at a time, the arms were severed from the disk and the aboral surface removed down to the level of the ambulacral spines. The whole structure was then carefully blotted with filter paper, especially the ambulacral groove. By placing the tip of the arm in a centrifuge cone and then slitting all the ampullae rapidly with a sharp scalpel it was possible to collect a small quantity of fluid by allowing it to run along the gutter formed by the remains of the ambulacral ossicles. Repeating this procedure on all five arms of a medium-sized animal of about 30 g weight, some 0·4–0·8 ml. of fluid could be obtained. Both samples were than centrifuged at 8000 *g* for 10 min to remove all corpuscles and any debris introduced during the sampling procedure and aliquots then removed for chemical analysis by the following methods. Sodium and potassium were estimated by means of an 'Eel' flame photometer after simple dilution (S.D. on ten determinations—1·5%), and calcium determined after separation as the oxalate and solution in 0·05 N perchloric acid. (S.D. on ten determinations—3%). Magnesium was estimated iodometrically after precipitation as the oxinate (S.D. on ten determinations—5%) and later, together with calcium by an EDTA titration with an 'Eel' titrator. (S.D. on ten determinations—2%). Chloride was determined by double decomposition with silver iodate, followed by iodometry as described by Milton & Waters (1949) (S.D. on ten determinations—1%), and sulphate after double decomposition with barium iodate as described by Webb (1939) (S.D. on ten determinations—2%). Total carbon dioxide was estimated by the microdiffusion technique of Conway (1957).

## IONIC COMPOSITION AND REGULATION

The results of previous analyses of echinoderm body fluids showed them to be almost identical, ionically, with the sea water in which the animals had been living, and they were thus suggested to be in physico-chemical equilibrium with it. With one major exception, this situation has been confirmed for both the perivisceral and ambulacral fluids of *A. rubens*. The exception is the potassium concentration within the water vascular system, which can be seen from Table 1 is some 60% up on the sea-water value. Admixture with perivisceral fluid or sea water obviously cannot be the origin of this enhanced value, but a further source of contamination must not be overlooked. During the sampling procedure the ambulacral fluid lies in contact with fragments of the muscular tube foot ampullae in the centrifuge cone. Potassium is present in this debris to the extent of some 103 mM/kg wet weight and leakage from the damaged tissue could perhaps be the source of this extra potassium. To examine the extent of any such leak, several samples of fluid were left in contact with macerated ampullae for periods of up to 1 h prior to centrifugation and analysis. No increase whatsoever in the potassium content of the

fluid was observed during this time and it was concluded that in this respect the sampling technique was a valid one.

Dialysis of these fluids against the sea water from which the animals had been taken led only to almost immeasurably small changes in the composition of the perivisceral fluid, but the potassium concentration in the ambulacral fluid fell to the sea water value with a half-time of 42 min with the system used. Small quantities of protein have been reported from the perivisceral fluid of echinoderms by several workers, but so far no data are available for the ambulacral fluid. Precipitation tests with trichloracetic acid, tannic acid,

TABLE 1. IONIC COMPOSITION OF THE BODY FLUIDS OF *ASTERIAS RUBENS* UNDER ENVIRONMENTAL CONDITION

| | | Perivisceral fluid | | Ambulacral fluid | |
|---|---|---|---|---|---|
| | Medium | | | | |
| Sodium | 429 mM/l. | 428 mM/l. | S.D. 5 mM | 418 mM/l. | S.D. 12 mM |
| Potassium | 9·5 | 9·5 | 0·4 | 15·1 | 1·5 |
| Calcium | 10·8 | 11·7 | 0·4 | 9·7 | 1·0 |
| Magnesium | 49·0 | 49·2 | 2·0 | 50·3 | 2·5 |
| Chloride | 494 | 487 | 12·0 | 481 | 18·0 |
| Sulphate | 25·4 | 26·7 | 0·5 | 25·5 | 0·4 |
| Carbon dioxide | 2·52 | 2·82 | 0·17 | — | — |
| pH | 7·8 | 7·2 | | 6·9 | |
| Protein | — | Tests negative | | Tests faintly positive | |

salicylsulphonic acid, and by boiling, all yielded negative results upon the perivisceral fluid, but were all faintly positive upon the ambulacral fluid. It was considered therefore, that the quantity was so small as to render its removal for analytical purposes unnecessary. In view of the small size of the protein concentration it was not considered feasible that a Donnan equilibrium could be responsible for the retention of the relatively large amount of potassium, and it is suggested that its presence within the water vascular system is due to an active accumulatory mechanism which may reside in the walls of the tube feet or ampullae.

It will also be seen from Table 1 that both the calcium and carbon dioxide concentrations in the perivisceral fluid are slightly above those of the medium. With the more acid pH of this fluid there are, as calculated from the data given by Harvey (1955), actually fewer carbonate ions present than in sea water, but even so, the solubility product for calcite is still not exceeded.

After reaching weight equilibrium in diluted sea water both the perivisceral and ambulacral fluids of *Asterias* have been shown to be isotonic with that medium. However, this should not be taken to mean that the relative ionic composition has remained unaltered. To examine this possibility, ten large animals of about 40–50 g weight were placed in each of a series of dilutions of sea water. In these media they were left for 50 h at 10° C, after which time they were removed, sampled and their body fluids analysed for the six most common ions. A similar experiment was performed upon small animals weighing between 2 and 5 g. Owing to the small quantities of fluid obtained

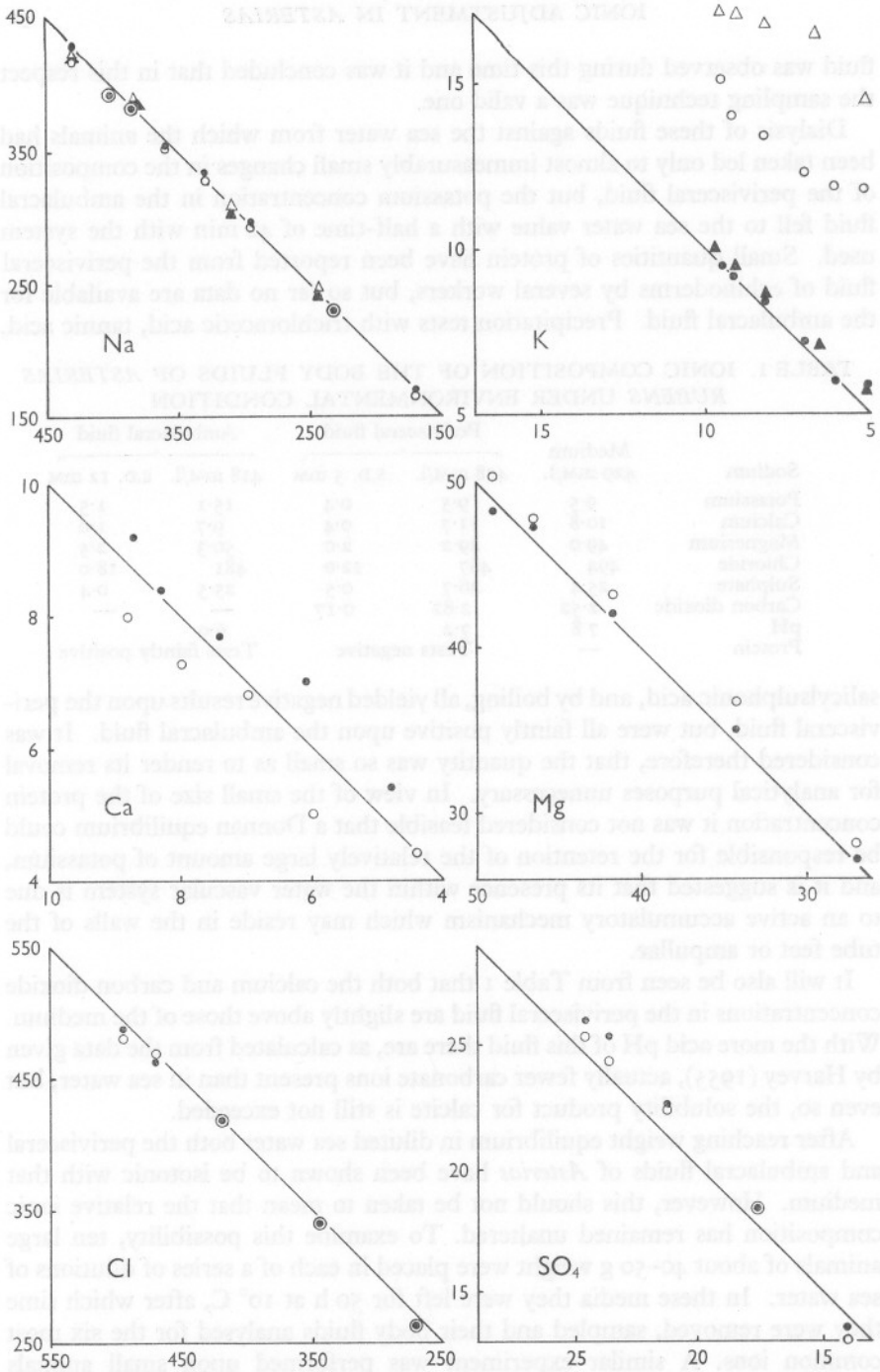


Fig. 1. Ionic regulation, in *Asterias rubens*, of sodium, potassium, calcium, magnesium, chloride, and sulphate. Horizontal axes—external concentration in mM/l. Vertical axes—internal concentration in mM/l. Solid line represents isotonicity. All points are the average of ten determinations on different animals. Coelomic fluid: large animals ●, small animals ▲. Ambulacral fluid: large animals ○, small animals △.

from these animals, especially from the water vascular system, only sodium and potassium were estimated in this instance. The results of these analyses are presented in Fig. 1, and indicate that in the perivisceral coelom only calcium is retained to any extent, the other ions attaining the same concentration as in the medium. In the water vascular system, however, the potassium concentration is effectively regulated down to at least 55% sea water which is well below the limit of salinity tolerance for North Sea animals. This situation is also true for small animals which possessed an even higher concentration of potassium in the water vascular system under environmental conditions than did the large ones and retained it to a greater degree in reduced salinities. The magnitude of the potassium accumulation is unaffected by temperature over the ecological range. This was demonstrated by keeping five large animals in 70% sea water at 0, 5, 10, 15 and 20° C. for 50 h before performing the analyses (see Table 2).

TABLE 2. THE EFFECT OF TEMPERATURE UPON THE RETENTION OF CERTAIN IONS IN *ASTERIAS RUBENS*

| Ion | Medium | 0° C | 5° C | 10° C | 15° C | 20° C |
|---|---|---|---|---|---|---|
| | | (i) Perivisceral fluid | | | | |
| Sodium | 330 mM/l. | 332 mM/l. S.D. 4 mM | 340 mM/l. S.D. 5 mM | 334 mM/l. S.D. 3 mM | 338 mM/l. S.D. 7 mM | 334 mM/l. S.D. 9 mM |
| Potassium | 7·0 | 6·9 S.D. 0·1 | 7·0 S.D. 0·1 | 7·2 S.D. 0·1 | 7·0 S.D. 0·2 | 7·1 S.D. 0·3 |
| Calcium | 8·0 | 10·1 S.D. 0·1 | 10·1 S.D. 0·5 | 10·1 S.D. 0·4 | 10·2 S.D. 0·6 | 10·0 S.D. 0·6 |
| | | (ii) Ambulacral fluid | | | | |
| Sodium | 330 | 320 S.D. 10 | 326 S.D. 7 | 328 S.D. 3 | 331 S.D. 8 | 330 S.D. 8 |
| Potassium | 7·0 | 12·8 S.D. 0·9 | 11·7 S.D. 1·0 | 11·8 S.D. 1·0 | 12·5 S.D. 1·5 | 12·7 S.D. 1·1 |
| Calcium | 8·0 | 8·0 S.D. 0·8 | 7·9 S.D. 1·1 | 7·4 S.D. 1·0 | 7·6 S.D. 1·0 | 6·8 S.D. 0·4 |

Although temperature seems to have no influence upon the somewhat limited powers of ionic regulation possessed by *Asterias*, the season of the year or state of the breeding cycle may not be without effect. Robertson (1939, 1949) found in his separate analyses of the ambulacral fluid of *Echinus esculentus* L. in March and September, that an enhanced potassium content was detectable only in March. He suggested that this difference may have been a reflexion of the reproductive cycle. To examine whether or not such a variation occurred in *Asterias*, ten large animals were collected from Whitstable at monthly, and during the breeding season fortnightly, intervals during the period October 1956 to September 1957. The results of the analyses performed upon the body fluids are presented in Figs. 2 and 3. No seasonal fluctuation in the concentration of potassium in the water vascular system was found, other than that due to salinity changes in the environment and the

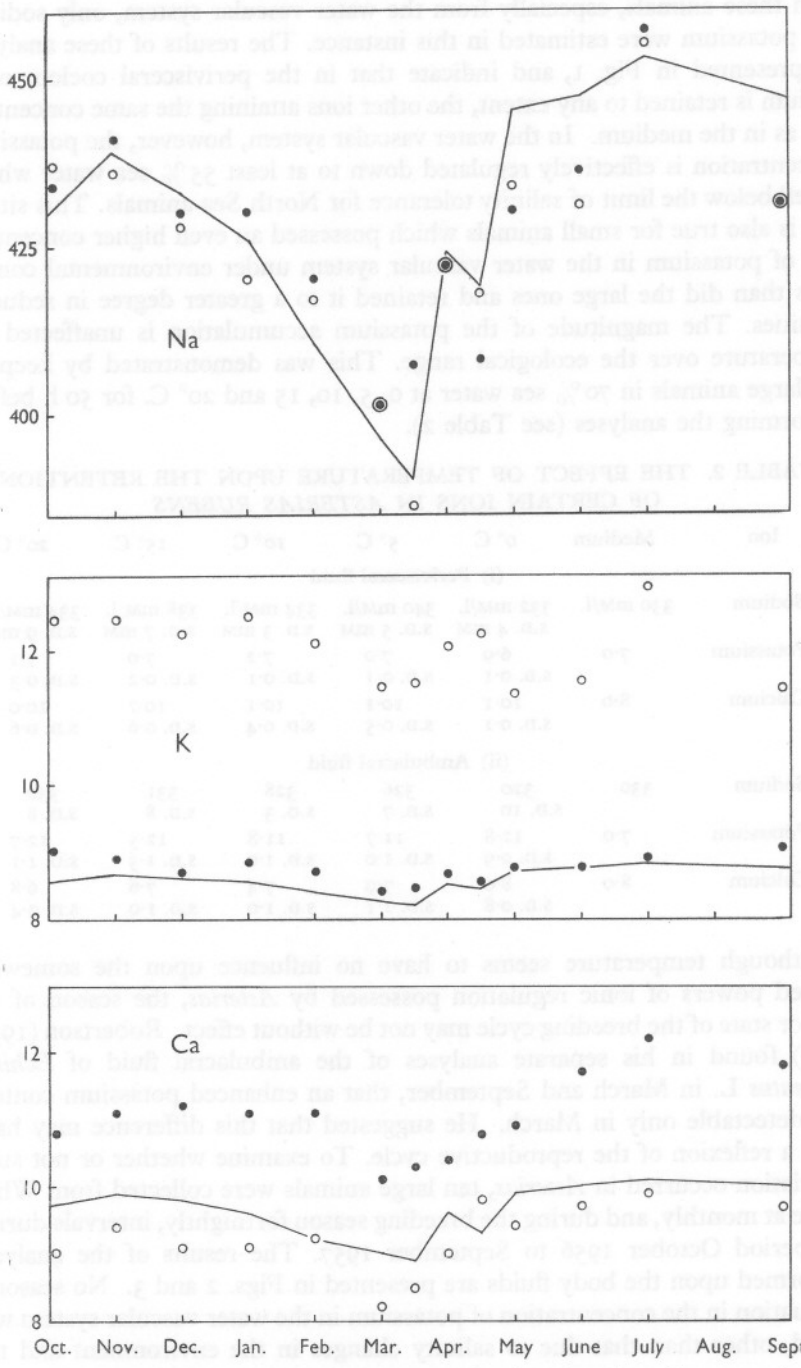


Fig. 2. Annual variation in the ionic composition of *Asterias rubens*, for sodium, potassium and calcium. Vertical axes—concentration in mM/l. Continuous line = sea-water variation, coelomic fluid ●, ambulacral fluid ○. All points are the average from ten animals.

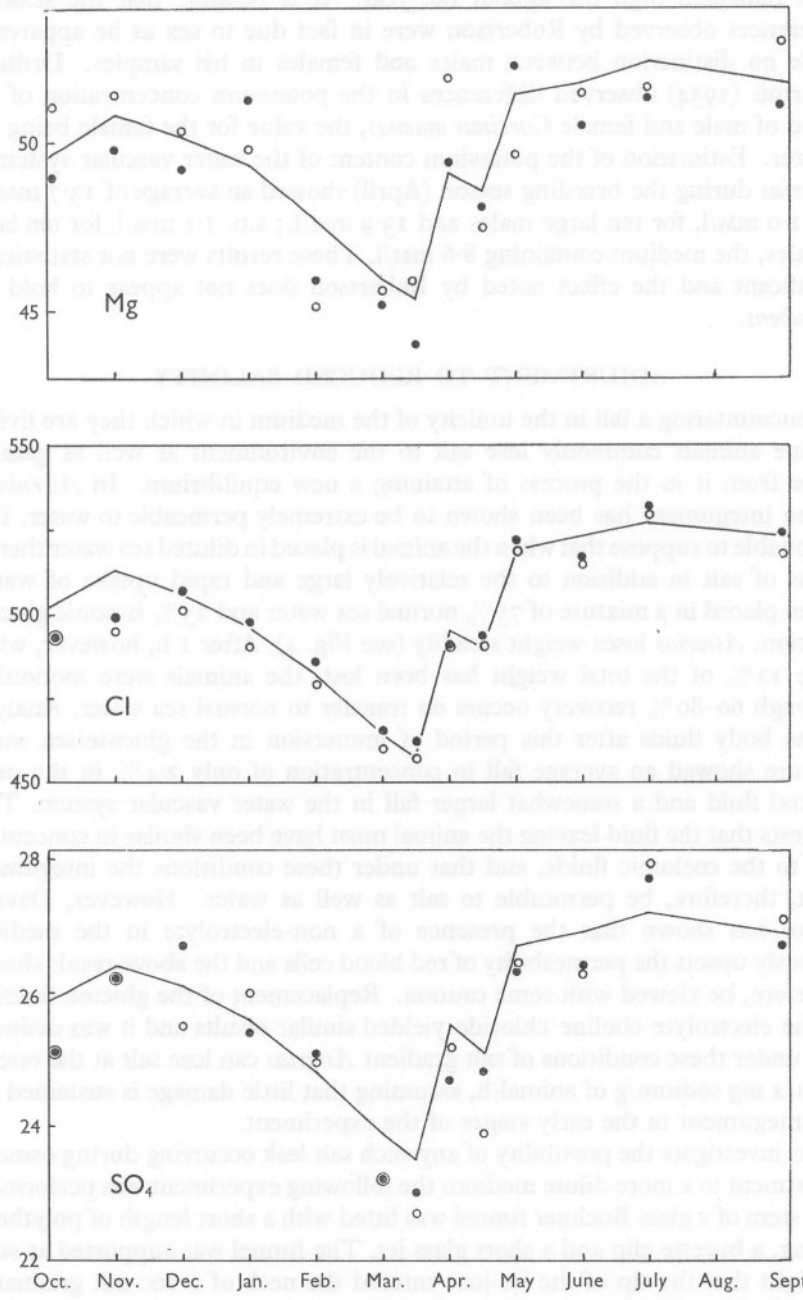


Fig. 3. Annual variation in the ionic composition of *Asterias rubens*, for magnesium, chloride and sulphate. Vertical axes—concentration in mM/l. Continuous line = sea-water variation, coelomic fluid ●, ambulacral fluid ○. All points are the average from ten animals.

level remained high throughout the year. It is possible that the seasonal differences observed by Robertson were in fact due to sex as he apparently made no distinction between males and females in his samples. Drilhon-Courtois (1934) observed differences in the potassium concentration of the blood of male and female *Carcinus maenas*, the value for the female being the greater. Estimation of the potassium content of the water vascular system of *Asterias* during the breeding season (April) showed an average of 13·7 mM/l.; S.D. 1·0 mM/l. for ten large males and 13·9 mM/l.; S.D. 1·1 mM/l. for ten large females, the medium containing 8·6 mM/l. These results were not statistically significant and the effect noted by Robertson does not appear to hold for *A. rubens*.

## ADJUSTMENT TO REDUCED SALINITY

On encountering a fall in the tonicity of the medium in which they are living, marine animals commonly lose salt to the environment as well as gaining water from it in the process of attaining a new equilibrium. In *A. rubens*, whose integument has been shown to be extremely permeable to water, it is reasonable to suppose that when the animal is placed in diluted sea water there is a loss of salt in addition to the relatively large and rapid uptake of water. When placed in a mixture of 75% normal sea water and 25% isotonic glucose solution, *Asterias* loses weight steadily (see Fig. 4). After 1 h, however, when some 12% of the total weight has been lost, the animals were motionless although 60–80% recovery occurs on transfer to normal sea water. Analysis of the body fluids after this period of immersion in the glucose/sea water mixture showed an average fall in concentration of only 2·4% in the perivisceral fluid and a somewhat larger fall in the water vascular system. This suggests that the fluid leaving the animal must have been similar in concentration to the coelomic fluids, and that under these conditions the integument must, therefore, be permeable to salt as well as water. However, Davson (1939) has shown that the presence of a non-electrolyte in the medium seriously upsets the permeability of red blood cells and the above result should therefore, be viewed with some caution. Replacement of the glucose fraction by the electrolyte choline chloride yielded similar results and it was deduced that under these conditions of salt gradient *Asterias* can lose salt at the rate of 1·0–1·2 mg sodium/g of animal/h, assuming that little damage is sustained by the integument in the early stages of the experiment.

To investigate the possibility of any such salt leak occurring during osmotic adjustment to a more dilute medium the following experiment was performed. The stem of a glass Buchner funnel was fitted with a short length of polythene tubing, a burette clip and a short glass jet. The funnel was supported at such a height that the tip of the jet just entered the neck of a 100 ml. graduated flask. With the clip closed, 100 ml. of 55% sea water were placed in the funnel. The clip was opened and the sea water allowed to run into the flask, the

funnel being allowed to drain for a few moments. Any deficit after such a transfer was made up with 55% sea water. This was repeated several times and after the apparatus had become thoroughly wetted, the change in volume on transfer was less than 0·1 ml. With the funnel containing 100 ml. of 55% sea water and the flask in position, a small starfish of about 14 g weight was rinsed quickly in 55% sea water, blotted and placed in the funnel for 1 h. After this time the clip was opened and the water allowed to drain back into the flask. The volume was usually about 98–99 ml. due to water having entered

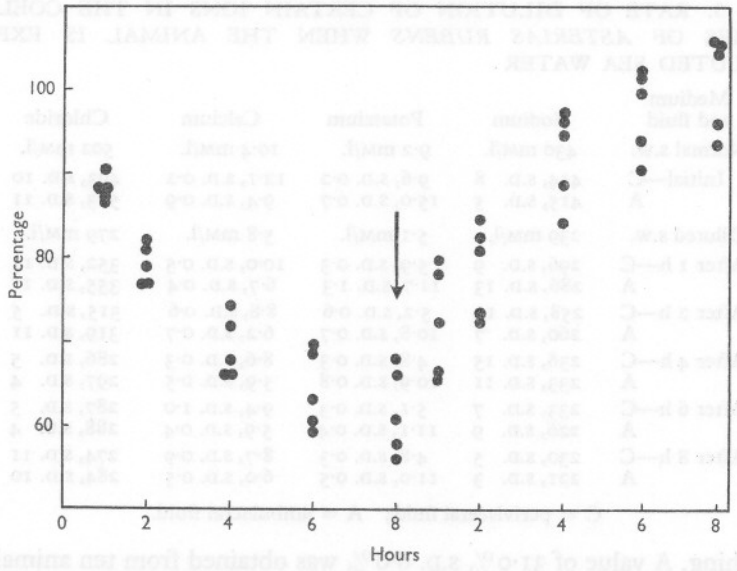


Fig. 4. Loss in weight by *Asterias rubens* in a mixture of 25% isotonic glucose and 75% sea water. The arrow indicates return to normal sea water.

the animal. If the flask was topped up to the mark with distilled water the content should still be at the same concentration as it was before the starfish was immersed in it. If it contained less salt then it would indicate that the animal had taken up salt as well as water which would worsen its position osmotically. If the sea water contained more salt it would indicate that the animal had lost salt in the process of adjustment. Estimations of the chloride content indicated a rise of about 1·6 mg chloride/g of animal/h, S.D. 0·5 mg Cl, based upon thirty determinations. Some of the results, however, did suggest that either there had been no net movement of salt at all, or that the animal had actually taken up a small amount, of the order of 0·1 mg. Despite the close correspondence to the value obtained in the last experiment, the variation from animal to animal is such that evidence of a consistent salt leak of any magnitude in *Asterias* during the process of osmotic adjustment to lowered salinity was not considered to have been demonstrated.

A possible explanation for this rather unexpected result can be sought in the next experiment. Twenty-five large animals were placed in a tank containing 50 l. of 55% sea water at 10° C. After intervals of 1, 2, 4, 6 and 8 h, five animals were removed, sampled and analysed for sodium, potassium, calcium and chloride in both the perivisceral and water vascular cavities. The results are presented in Table 3. Secondly, the volume of the perivisceral cavity was estimated by weighing an animal, making a slit along the aboral surface of each of the arms and allowing it to drain for about 15 min before

TABLE 3. RATE OF DILUTION OF CERTAIN IONS IN THE COELOMIC CAVITIES OF *ASTERIAS RUBENS* WHEN THE ANIMAL IS EXPOSED TO DILUTED SEA WATER

| Medium and fluid | Sodium | Potassium | Calcium | Chloride |
|---|---|---|---|---|
| Normal s.w. | 430 mM/l. | 9·2 mM/l. | 10·4 mM/l. | 502 mM/l. |
| Initial—C | 414, S.D. 8 | 9·6, S.D. 0·2 | 12·1, S.D. 0·2 | 493, S.D. 10 |
| A | 415, S.D. 5 | 15·0, S.D. 0·7 | 9·4, S.D. 0·9 | 503, S.D. 11 |
| Diluted s.w. | 239 mM/l. | 5·1 mM/l. | 5·8 mM/l. | 279 mM/l. |
| After 1 h—C | 296, S.D. 9 | 5·9, S.D. 0·3 | 10·0, S.D. 0·5 | 352, S.D. 11 |
| A | 286, S.D. 13 | 11·7, S.D. 1·3 | 6·7, S.D. 0·4 | 355, S.D. 11 |
| After 2 h—C | 258, S.D. 12 | 5·2, S.D. 0·6 | 8·8, S.D. 0·6 | 315, S.D. 5 |
| A | 260, S.D. 7 | 10·8, S.D. 0·7 | 6·2, S.D. 0·7 | 319, S.D. 11 |
| After 4 h—C | 236, S.D. 15 | 4·8, S.D. 0·3 | 8·6, S.D. 0·3 | 286, S.D. 5 |
| A | 233, S.D. 11 | 10·9, S.D. 0·8 | 5·9, S.D. 0·5 | 297, S.D. 4 |
| After 6 h—C | 233, S.D. 7 | 5·1, S.D. 0·3 | 9·4, S.D. 1·0 | 287, S.D. 5 |
| A | 226, S.D. 9 | 11·1, S.D. 0·4 | 5·9, S.D. 0·4 | 288, S.D. 4 |
| After 8 h—C | 230, S.D. 5 | 4·8, S.D. 0·3 | 8·7, S.D. 0·9 | 274, S.D. 11 |
| A | 221, S.D. 3 | 11·0, S.D. 0·5 | 6·0, S.D. 0·5 | 284, S.D. 10 |

C = perivisceral fluid; A = ambulacral fluid.

reweighing. A value of 31·0% S.D. 6·0% was obtained from ten animals. By making the assumption that all the water entering the animal during the early stages of adjustment to reduced salinity remains in the perivisceral coelom, it is possible to calculate, from a knowledge of the weight increase in that medium, the theoretical rate of dilution of the ions in the coelomic fluid. Measurements made of the change of volume of the coelomic cavity after immersion for 1 h in 55% sea water suggests this assumption to be a reasonable one. The solid line in Fig. 5 indicates the theoretical rate of dilution of sodium in an animal immersed in 55% sea water, the circles representing the actual values found by analysis. Similar figures may be drawn for the other ions investigated. The close correspondence between the theoretical dilution rate and that determined experimentally suggests that when placed in a diluted medium *Asterias* takes in water sufficiently rapidly to reduce the magnitude of any salt exchange to negligible proportions. The small discrepancy is probably due to the first assumption not being completely valid, for it seems obvious that the tissues themselves must take up some water. Analysis of the aboral integument of animals under environmental conditions

and after 1 h in 55 % sea water shows there to be a fall in sodium concentration from 273 mM/kg, S.D. 8 mM/kg to 182 mM/kg, S.D. 23 mM/kg, both measurements being the average from five animals. This could be the result of both salt loss and water uptake, for the integument takes on a softer and more gelatinous appearance after some time in a more diluted medium. This was also noticed by Schlieper (1957) for animals normally living in conditions of reduced salinity.

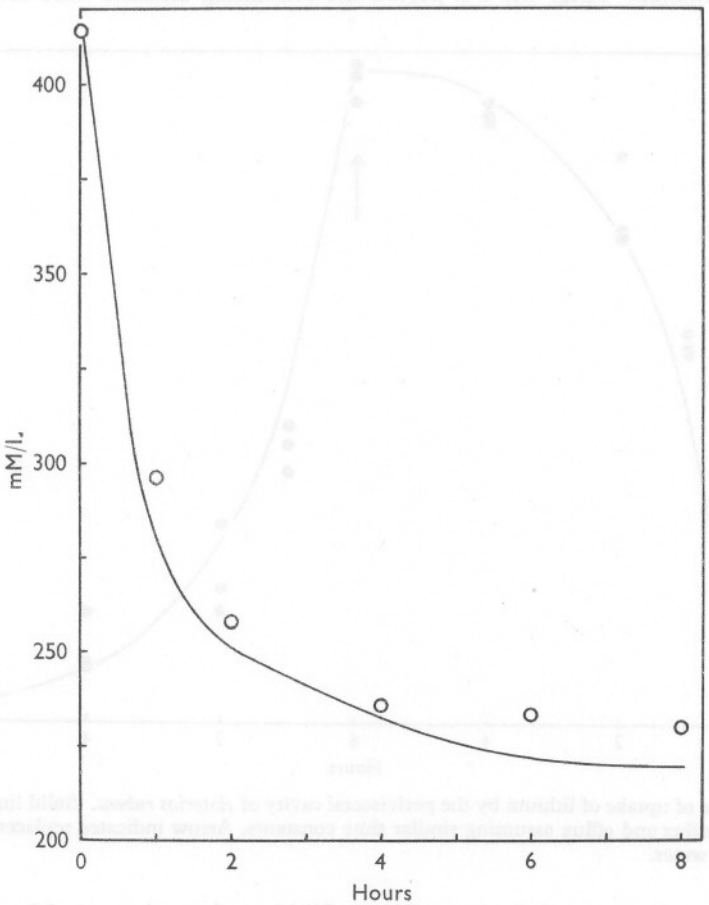


Fig. 5. Theoretical and observed rates of dilution of sodium in the perivisceral cavity of *Asterias rubens* when the animal is immersed in 55 % sea water. Solid line is the calculated rate.

Bethe & Berger (1931*b*), found variations in the rate of uptake of iodide by *Echinus esculentus* when different regions of the integument were sealed. A differential permeability to various ions was observed by Koizumi (1936) in the holothurian *Caudina chilensis*. To investigate a little further the perme-

ability of the integument of *Asterias rubens*, some experiments were conducted with the aid of lithium. This element was chosen because it is similar in many of its properties to sodium and potassium, it is relatively easy to detect in small amounts with a flame photometer and it does not occur in significant quantities in sea water. Twenty-four animals were placed in a tank of normal sea water containing 208 mg/l. of this ion. After 1, 2, 4 and 6 h, three animals were removed and the lithium content of the perivisceral and water vascular fluids estimated. After the 6 h period the remaining animals were returned

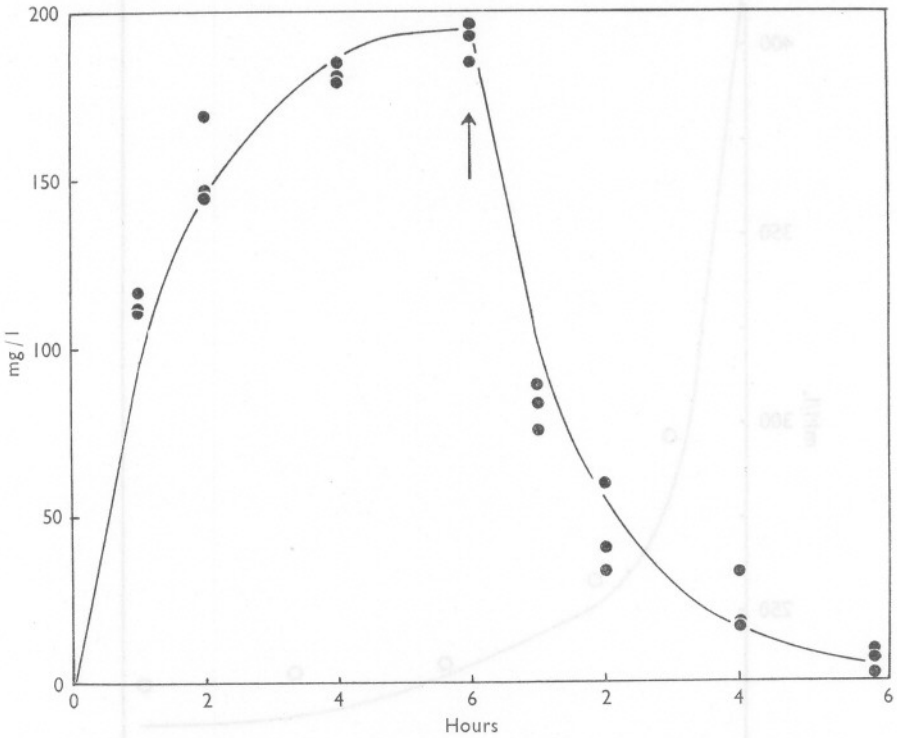


Fig. 6. Rate of uptake of lithium by the perivisceral cavity of *Asterias rubens*. Solid line is the calculated influx and efflux assuming similar time constants. Arrow indicates replacement in normal sea water.

to normal sea water and the rate of loss of lithium from these cavities determined. The values for the perivisceral cavity are indicated by the points in Fig. 6. The continuous line for the efflux was obtained by drawing a straight line through the data when the logarithms of the concentrations had been plotted against time. The continuous line for the influx is the time course of entry to be expected if this took place exponentially and with the same time constant as the efflux. The good agreement between the influx data and the

theoretical line shows that the permeability coefficients for lithium influx and efflux are very close. In Table 4 the data for the water vascular system and perivisceral coelom are compared and it is clear that the time course for lithium movements into both these cavities is very similar. This is surprising since the volume of the perivisceral cavity is many times larger than that of the water vascular system, and the membranes bounding them are very different in nature. One must either regard the similar time courses for lithium movement into these two cavities as a fortuitous phenomenon or, and this seems more reasonable, one must suppose that the ampullary wall separating the two cavities is relatively more permeable to lithium than the outer walls of

TABLE 4. RATE OF UPTAKE OF LITHIUM BY *ASTERIAS RUBENS*

| Time | Conc. in perivisceral fluid | | Conc. in ambulacral fluid | |
|---|---|---|---|---|
| Concentration in the experimental medium = 208 mg/l. | | | | |
| Initially | — | | — | |
| After 1 h | 117, 111, 112 | Average = 113 | 111, 115, 115 | Average = 114 |
| After 2 h | 145, 147, 169 | Average = 154 | 159, 142, 135 | Average = 145 |
| After 4 h | 179, 181, 185 | Average = 182 | 175, 181, 188 | Average = 181 |
| After 6 h | 185, 193, 196 | Average = 191 | 184, 186, 170 | Average = 180 |
| Animals returned to normal sea water—no lithium | | | | |
| After 1 h | 75, 83, 89 | Average = 82 | 80, 85, 89 | Average = 85 |
| After 2 h | 33, 40, 59 | Average = 44 | 32, 41, 56 | Average = 43 |
| After 4 h | 17, 18, 33 | Average = 23 | 21, 32, 32 | Average = 28 |
| After 6 h | 2, 7, 9 | Average = 6 | 2, 5, 8 | Average = 5 |

either cavity. Furthermore, it is quite clear that the mechanism for accumulating potassium in the ambulacral fluid can distinguish between lithium and potassium and this adds weight to the hypothesis that this latter ion is present as a result of an active process.

Much of this work was carried out during the tenure of a Research Assistantship at Sir John Cass College, London. My thanks are due to Professor J. E. Smith, F.R.S., for his supervision of the thesis of which some of this paper formed a part, and for the use of his department's marine laboratory at Whitstable. I am also grateful to the Director of the Plymouth Laboratory of the Marine Biological Association for his hospitality whilst I occupied the London table there, and to various members of his Staff for much helpful advice. Part of the travelling expenses and the purchase of the flame photometer and centrifuge were met by a grant from the Central Research Fund of London University.

## SUMMARY

The perivisceral and ambulacral fluids of *Asterias rubens* have been shown to be not only isosmotic but isoionic with sea water, even if that medium is diluted by nearly half. However, there is a slight accumulation and regulation of calcium in the perivisceral fluid and to a much more marked extent,

potassium in the water vascular system. The rapidity with which the potassium diffuses away when the ambulacral fluid is dialysed against sea water suggests that its presence in the water vascular system is due to an active accumulatory mechanism. This mechanism is capable of functioning when the animal encounters sea water of significantly reduced salinity, over the temperature range 0–20° C and extends throughout the season apparently uninfluenced by the breeding cycle or sex of the animal. Smaller animals tend to have a higher concentration of potassium in the water vascular system than do larger ones. Direct and indirect measurements of salt loss under various conditions, together with observations on the rate of lithium transfer, suggests that the integument is very permeable to many ions as well as to water. This supports the hypothesis that the ionic regulation observed results from an active process and not merely from an impermeability of the integument, although measurements with potassium itself have not yet been made.

Salt loss during osmotic adjustment to a more dilute medium is small and variable, on occasions there even being a slight uptake. It is suggested that this is due to the animal taking in water so rapidly that the concentration in the coelomic cavity falls to that of the outside medium before any appreciable leakage occurs.

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