J. Exp. Biol. (1963), **40**, 87–104
With 7 text-figures
Printed in Great Britain

OSMOTIC REGULATION IN THE BRACKISH WATER TELEOST, *BLENNIUS PHOLIS*

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(*Received* 23 *July* 1962)

INTRODUCTION

The marine teleosts have a blood concentration much lower than that of the surrounding sea water and they maintain a constant composition of their internal medium by special regulatory mechanisms. Essentially, the osmotic problem of the marine teleost is to obtain enough water to replace that lost by osmotic flow through the gills. It is known that their blood electrolyte concentration is maintained at an almost constant level by continual drinking of sea water and simultaneous excretion of ions, mainly through non-renal routes.

Mullins (1950) studied various aspects of the osmotic regulation of sticklebacks (*Gasterosteus aculeatus*) obtained from brackish water. The fish were kept either in Baltic sea water (*ca.* 230 mM./l. Na) or in artificial sea water of the same salinity. In addition he used tap water or diluted sea water as fresh water (*ca.* 3 mM./l. Na) in experiments principally with the isotope ^{24}Na. Upon transferring the fish from fresh water to sea water he found the rate of drinking was approximately 10 mM. Na/kg. fish/hr., or 4 % of the body weight/hr.

Several other brackish-water fish exhibit great osmo-regulating ability. The blenny (*Blennius pholis*) is a littoral marine teleost which is likely to be exposed to considerable change in the osmotic concentration of its external environment, for it is found in rock pools and river mouths. This paper records the results of experiments designed to measure, first, the influxes and effluxes of sodium in the blenny in various concentrations of sea water by use of the isotopic tracer ^{24}Na, and, secondly, the electric potential differences across the gills and the ionic concentrations in blood serum in these external media.

THEORETICAL BASIS

In the circumstances where the ionic composition of an animal is maintained in a steady state with the surrounding medium, the influx from the external medium is exactly balanced by a similar efflux from the animal. The flux can be expressed as mM. of ion leaving or entering per kg. fish (or litre blood) per hr.

In the discussion which follows the animal is considered to be a single-compartment system. This is justified because in the flux experiments sodium is the ion which is studied and this ion is known to be found predominantly in the extracellular fluid of the animal.

Consider the case where an amount of a radioactive ion is added to the external medium and the steady state is unaltered by this quantity of isotope. The activity of the animal's blood should rise and tend asymptotically to an equilibrium value. It can be shown that the activity, A (counting rate/unit volume) of the blood at any time t hours after addition of the tracer ion to the medium is given by

$$A = \frac{C_i}{C_o} B \left[1 - \exp\left(\frac{-kt}{C_i}\right)\right],$$

where B is the activity of the medium, C_o is the concentration (mM./l.) of ion in the medium, C_i is the concentration (mM./l.) of ion in the blood, and k is the total flux (mM. ion/l. blood/hr.) of the ion. Hence A_∞, the activity of the blood, when the exchange is complete, is given by $A_\infty = C_i B/C_o$, i.e.

$$A = A_\infty \left[1 - \exp\left(\frac{-kt}{C_i}\right)\right].$$

From values of A at known times k can be calculated, or, alternatively, on plotting $\log_{10}[A_\infty/(A_\infty - A)]$ against t a straight line should be obtained and the slope of this line is given by $k/2{\cdot}3C_i$.

The course of the exchange need not be followed for a great length of time because A_∞ can be calculated from $C_i B/C_o$.

Values of k, the total ion flux, can also be obtained from experiments which are the reverse of the above. The animal is placed in the radioactive medium for some time. Then the efflux of the radioactive ion is observed in an inactive medium of 'infinite' volume.

In this case the activity A is given by

$$A = A_0 \exp\left(\frac{-kt}{C_i}\right),$$

where A_0 is the activity at zero time, i.e. time of removal from the active medium. On plotting $\log_{10} A$ against t a straight line should be obtained of slope $[-k/2{\cdot}3C_i]$. Thus k can be found from this slope.

Measurements of this kind give the total influx and efflux of ion, but it is also valuable to know the passive component of efflux and influx of ion before one attempts to establish 'active transport' of the ion. In a steady state the presence of an ion at a different electrochemical potential inside the animal, i.e. in the blood, proves that work must be done on that ion to maintain the different electrochemical potential and there is an 'active transport' of the ion.

Ussing (1950) and Teorell (1949) have shown that the passive independent movement of ions across membranes is governed by the relation

$$\frac{J_{\text{in}}}{J_{\text{out}}} = \frac{C_o}{C_i \exp(z_j FE/RT)},$$

where J_{in} is the passive influx across the membrane expressed in mole cm.$^{-2}$ sec.$^{-1}$, J_{out} is the passive efflux across the membrane in mole cm.$^{-2}$ sec.$^{-1}$, C_o is the concentration (mole cm^{-3}) of ion j on the outside of the membrane, C_i is the concentration of ion j on the inside, z_j the algebraic valency of the ion, F is the Faraday, E is the electric

potential difference in volts across the membrane, R is the gas constant and T is the absolute temperature. This expression follows from the fact that the ratio of passive fluxes is proportional to the electrochemical activity ratio.

Writing,

$$\frac{J_{\text{in}}}{J_{\text{out}}} - 1 = \frac{J_{\text{in}} - J_{\text{out}}}{J_{\text{out}}} = \frac{\text{net passive influx}}{J_{\text{out}}}$$

then,

$$J_{\text{out}} = \frac{\text{net passive influx}}{[C_o - C_i \exp(z_j FE/RT)]/C_i \exp(z_j FE/RT)}.$$

It is possible with the Goldman, or constant field theory, which makes the simplifying assumption that the electric potential gradient within the membrane is linear, to calculate the net influx of any ion, and this has been solved by Hodgkin & Katz (1949). For sodium the net influx, J_{Na} moles cm.$^{-2}$ sec.$^{-1}$, is given by

$$J_{\text{Na}} = \frac{P_{\text{Na}}[FE/RT]}{1 - \exp(FE/RT)} \cdot [[\text{Na}_o] - [\text{Na}_i] \exp(FE/RT)],$$

where $[\text{Na}_o]$ is the sodium concentration on the outside of the membrane in moles cm.$^{-3}$, and $[\text{Na}_i]$ is the sodium concentration on the inside of the membrane. In this equation P_{Na} has been written for $U_{\text{Na}} RTk_{\text{Na}}/a$ where $k_{\text{Na}} = C'_{\text{Na}}/C_{\text{Na}}$ is the ion partitition coefficient between the membrane and the solution (i.e. with a concentration C_{Na} of sodium on the outside of the membrane then the concentration of sodium within the membrane at the phase boundary will be $k_{\text{Na}}\, C_{\text{Na}} = C'_{\text{Na}}$), U_{Na} is the mobility of sodium inside the membrane in cm. sec.$^{-1}$ joule^{-1} cm. mole^{-1}, and a is the membrane thickness. It is assumed that P_{Na} does not vary with changes in $[\text{Na}_o]$ and $[\text{Na}_i]$.

Hence the passive efflux, J_{out}, is given by

$$J_{\text{out}} = \frac{P_{\text{Na}}[FE/RT]}{1 - \exp(FE/RT)} \cdot [[\text{Na}_i] \exp(FE/RT)].$$

An expression for the passive influx J_{in} can be derived similarly.

In this analysis 'exchange diffusion', as described by Ussing (1952), has been neglected and, if this process predominates in the gill membranes, the passive fluxes will be independent of the membrane potential, E, and of the concentrations of the ion on both sides of the membrane when all the 'carriers' in the membrane are saturated. In this case the exchange flux will be limited by the number of 'carriers' and their rate of movement across the membrane. In such a model the ratio of passive fluxes will tend to unity.

MATERIALS AND METHODS

Adult and young *Blennius pholis* were used. These were obtained from Black Rocks, Penmon, Anglesey, the Marine Station, Plymouth and Port Seton, the Firth of Forth. All flux experiments were conducted in the range 17–20° C. and at 8° C., while the potential measurements were made in the range 14–19° C.

The isotope ^{24}Na was obtained from Amersham in the form of neutron-irradiated NaCl in sterilized saline isotonic with plasma (*ca.* 160 mM. Na/l.) (pH 6–8). The

counting equipment was an end-window G.M. counter mounted in a lead castle, with power pack, probe unit and scaler. A Mullard liquid G.M. tube was used in certain experiments. The potential difference (P.D.) measurements were made with two calomel electrodes, and a high impedance null-reading millivoltmeter (PHM3, Radiometer, Copenhagen).

Ionic concentrations in blood

The electrolyte levels in the blood were always measured on the conclusion of the potential measurements in different media. After removing the animal from the medium it was killed by a sharp blow on the head, washed quickly with de-ionized water, dried on filter paper and pinned to a wooden block. Collecting pipettes had been made by drawing out 2 mm. diameter glass tubing to a sharp tip and mounting each pipette on a piece of rubber tubing the other end of which was held in the mouth. The body wall was cut along the ventral surface from posterior to anterior and the heart punctured by the fine tip of the pipette. Blood rose into the tip by capillary attraction and a small negative pressure was applied by mouth. Finally, some liquid paraffin was drawn into the pipette, the tip was sealed with wax and the sample was centrifuged. Samples of blood serum were drawn off from below the paraffin layer into a micro-pipette and suitably diluted for measurement against standard NaCl and KCl solutions using an EEL flame-photometer. In this way duplicated sodium and potassium concentrations in blood serum of the animals were determined. The accuracy of the sodium concentrations was limited by the reading error on the instrument (maximum error was about 5 %) and possible dilution of samples during withdrawal from the animals. The accuracy of the potassium measurements was exceedingly low due to almost inevitable haemolysis of red cells during centrifugation, and, for this reason, these results are not quoted in detail.

The chloride concentrations in blood serum were measured by the direct titration of serum volumes (diluted with 5 % nitric acid) with 0·01 M silver nitrate solution and the end-point was determined potentiometrically. This method was originally used by Sanderson (1952) and modified successfully for smaller samples by Ramsay, Brown & Croghan (1955) and Croghan (1958).

Whole-animal samples

Several animals were removed from the medium, killed quickly as before, rinsed rapidly with de-ionized water, dried with filter paper and weighed. These were then dissolved individually in 10 ml. of concentrated nitric acid and made up to a known volume with de-ionized water after filtration. These samples were then suitably diluted and used along with a standard sodium chloride solution with the flame-photometer to give the whole-body sodium content of the animals.

The water content of the animals was determined by removing animals from the medium, killing them, drying with filter paper and weighing them. After drying in an oven at 100° C. for several hours the animals were reweighed.

Efflux experiments

Effluxes were measured both by monitoring animals mounted in a current of inactive washing medium under an end-window counter, and by measuring the activity of the effluent itself. The former method gave more accurate values of the total flux k, but the latter gave more information about sudden changes in k when the washing medium was changed.

In these former experiments the animals were transferred to about 500 ml. of sea water or suitable dilutions of sea water, to which had been added approximately 3 mc. of ^{24}Na, and left for several hours to equilibrate with the radioactive component of the medium. The radioactive medium was aerated throughout the 'loading' of the fish. Upon removal each animal was washed rapidly with inactive sea water and placed in a piece of polythene tubing of 12 mm. diameter for the adults and 5 mm. diameter for the young. This tubing was then placed in a piece of Pyrex glass tubing of 15 mm. diameter which was clamped in a V-saddle under the counter window with the animal facing the direction of washing flow. Wire gauze was welded on two ends of two pieces of polythene tubing by heat; one piece of this tubing led into the Pyrex glass holder from a reservoir of the washing medium, while the other piece carried the effluent from the holder to a waste bottle. The wire gauze on the ends of the tubing ensured that the animal could not escape and also helped to keep the animal in position under the counter window. A brass filter (1 g./cm.^{2}) was placed between the counter and the animal to eliminate the Beta radiation from the ^{24}Na, which could cause variation in the counting rate due to possible change in the thickness of the layer of medium above the animal. The advantages of this method were that there was no need to take blood samples and the washing medium could be changed easily. After counting, the animal was returned to a large volume (10 l.) of the medium. During counting-rate determinations the animals were washed with the inactive medium passing through the holder continuously (*ca.* 10 ml./min.).

In the experiments conducted at 8° C. the washing medium was maintained at this temperature by suitable positioning of crushed ice around the vessels containing the medium.

In the other efflux experiments the animals were 'loaded' in an active 40% sea water (s.w.) solution, and then removed and washed rapidly with the inactive medium. The animals were transferred to a known volume of the inactive medium and at certain time intervals 10 ml. samples were pipetted from the washing solution, which was continuously mixed by bubbling air through it. After a known time the animals were removed, washed with the inactive medium and transferred to inactive sea water of known volume. Again 10 ml. samples of this continuously mixed medium were removed at certain time intervals. The samples were afterwards counted in a liquid G.M. tube.

All counting rates were corrected for background and radioactive decay. Where the logarithmic function of the counting rate was plotted against time the slopes of the straight lines were corrected for the decay. In the whole body counting there was no need to correct for the dead time (*ca.* 500 μsec.) as the counting rate was never greater than 1000 counts/min.

Sources of error

There were two sources of error in the whole-body counting rates of the fish. The maximum statistical error in the counting rate was 5 %; the second source of error was the possible change in position of the fish under the counter window during counting. To estimate the percentage error in the counting rate due to a change in position parallel to the direction of flow of the washing medium, a fish 'phantom' was made from paraffin wax and loaded with a suitable quantity of active sea water. With the usual counting conditions and assuming a maximum total movement of 1 cm. on either side of the central position the percentage error in the counting rate was 8·2 %. Thus the total maximum error in the counting was 10 %.

The errors involved in the experiments with the liquid G.M. tube are shown in Figs. 6 and 7.

Influx experiments

The animals were immersed in an active medium and removed at various time intervals. After killing they were rinsed with de-ionized water and blood samples were obtained as above and delivered to weighed planchets, which were reweighed and dried on a hot plate (60° C.). Samples of the active medium were prepared in the same way and both samples were counted under the same conditions. The maximum statistical error in the counting rate was 3 %.

Potential measurements

In these experiments the external media were prepared by suitable dilutions of an artificial 100 % S.W. (chief constituents were 470 mM. sodium, 549 mM. chloride and 10 mM./l. potassium). The 150 % S.W. was prepared by adding 235 mM. sodium chloride to a litre of 100 % S.W. as in the efflux experiments. Adult animals were used in these experiments and their wet weights lay within the range 2·5–7 g.

The animals were individually transferred from a large well-aerated polythene tank of sea water to the new external media in glass jars which were also continuously aerated. The animals were left for at least 48 hr. to acclimatize before the potential measurements were made. During the potential measurements the animal was clamped by the tail fin with its head and gills immersed in the medium (see Fig. 1), which was continuously aerated and circulated between two polythene vessels. The problem of holding the animal was solved by placing the animal in a vertically clamped 3 cm. diameter thin Perspex cylinder and filling the space between the animal and cylindrical surface above the head with cork. This ensured that there was little movement of the animal and at the same time was unlikely to cause damage to the gills. The Perspex cylinder was drilled with many holes to ensure adequate circulation of the aerated medium around the head region of the animal.

Micro-electrode tips were made by drawing out small pieces of 2 mm. diameter glass tubing to a sharp point and were filled with 3 M-KCl. One of these fine tips was attached by a rubber collar to the tip of a Pye Hg/Hg_2Cl_2/sat. KCl electrode and the P.D. between this and another Hg/Hg_2Cl_2/sat. KCl electrode, clamped in the external medium, was measured with a Radiometer pH Meter 3. The P.D. was measured with both electrodes in the external solution to determine any asymmetry. Then the tail of the animal was sprayed several times with de-ionized water and blotted dry to

reduce short-circuiting. The fine electrode was mounted on a Prior micro-manipulator and the tip was inserted under the skin of the tail region where it was assumed to be in contact with the body fluids; the P.D. was then measured at various time intervals. There was always some uncertainty as to whether the tip was in the blood, but on numerous occasions blood was observed at the point of insertion. This method was thought to have an advantage over measurements of P.D. across isolated gills because of the increased likelihood of short-circuiting in the latter method. On completion of the experiment the P.D. between the electrodes was again measured in the medium

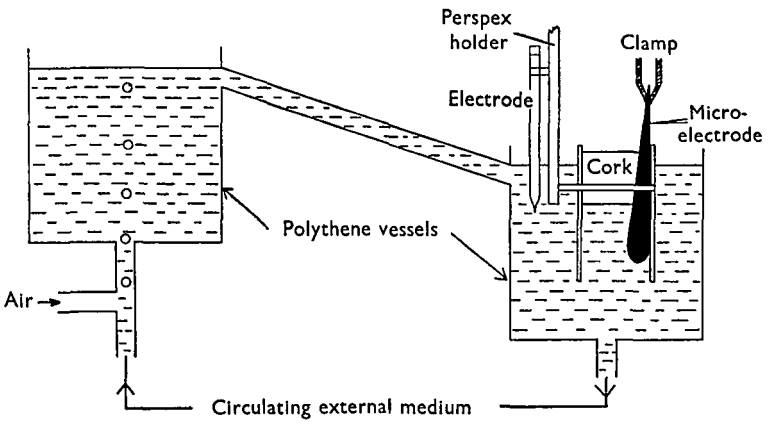

Fig. 1. The apparatus for the measurement of electric potential differences between blood and external medium.

and a correction applied to the observed steady value of the P.D. between blood and medium. In this way the P.D. across the gill membranes was measured in 10, 40, 100 and 150 % S.W.

In all experiments the P.D. could be measured to at least ±0·5 mV. The sign convention chosen in this paper for the statement of P.D.s is that the potential of the extracellular fluid of the animal has always been measured with the external medium as reference. The Nernst potentials have also been stated as the potential of the inside of the animal with respect to the external medium.

RESULTS

The results of the determinations of the sodium and chloride concentrations in the blood serum are shown in Fig. 2. The total body sodium content of three fish in 100 % S.W. was found to have a mean value ± S.E. of 57·8 ± 1·7 mM. Na/kg. fish (wet weight). The water content of six fish was found to have a mean value ± S.E. of 80·9 ± 0·9 % wet weight. Taking the blood sodium concentration as 170 mM. Na/l., the total body sodium as 60 mM. Na/kg. fish and the water content as 80 % of the wet weight, this would mean that the extracellular fluid ('sodium space') was approximately 45 % of the water content of the fish.

Efflux experiments

The efflux of sodium from the blenny was measured in different concentrations of the external medium. Sea water was diluted either with tap water (*ca.* 0·1 mM. Na/l.)

or de-ionized water. Several efflux measurements were made in 100 % S.W. and the total flux k was determined in mM. Na/l. blood/hr. In 40 % S.W. the animal was in an approximately iso-osmotic medium and efflux of sodium was assumed to be by passive diffusion only. Several additional efflux measurements were made in 10 % S.W. The values of the total fluxes k for these media are shown in Fig. 3.

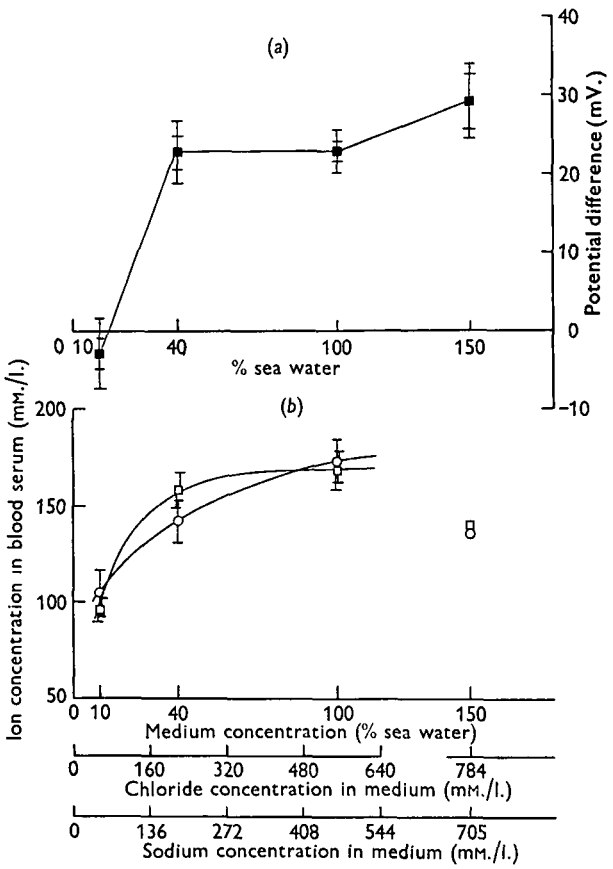

Fig. 2. (*a*) The relation of potential difference between blood and medium in animals, adapted to various concentrations of external medium. (*b*) Sodium and chloride concentrations in blood serum. In (*a*) the mean potential difference ■ is shown with S.E. and S.D. In (*b*) sodium concentration □ and chloride concentration ○ are given with S.E. It should be noted that, for concentrations of the external medium greater than 100 % S.W., the chloride concentration axis is not linear because the sodium concentration was increased proportionately to give 150 % S.W. by adding a suitable quantity of sodium chloride.

To observe the change in the efflux rate when the animal was changed from 40 to 100 % S.W. the animal was 'loaded' in an active 40 % S.W. solution (*ca.* 2 mc. in 200 ml.), and the effluxes were measured when the animal was perfused, first with 40 % S.W., then with 100 % S.W. and finally with 40 % S.W. (see Fig. 4). This experiment showed that the efflux of sodium increases very rapidly after transfer to more concentrated sea water. It also confirmed that there was little damage to the gills during the counting operation, because the efflux rates in 40 % S.W. initially and finally

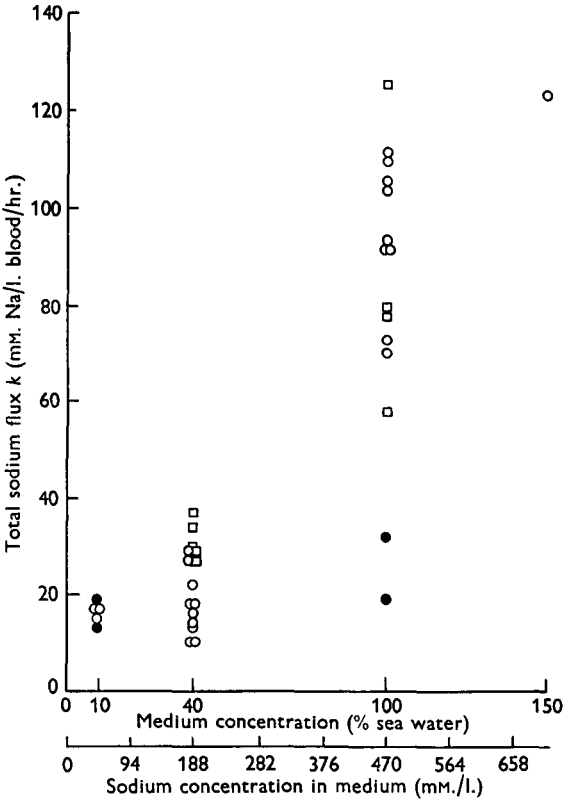

Fig. 3. The total sodium fluxes in animals adapted to different external media. Total effluxes ○ at room temperature (17–19° C.) and total effluxes ● at 8° C. are given. Total influxes □ at room temperature are also given.

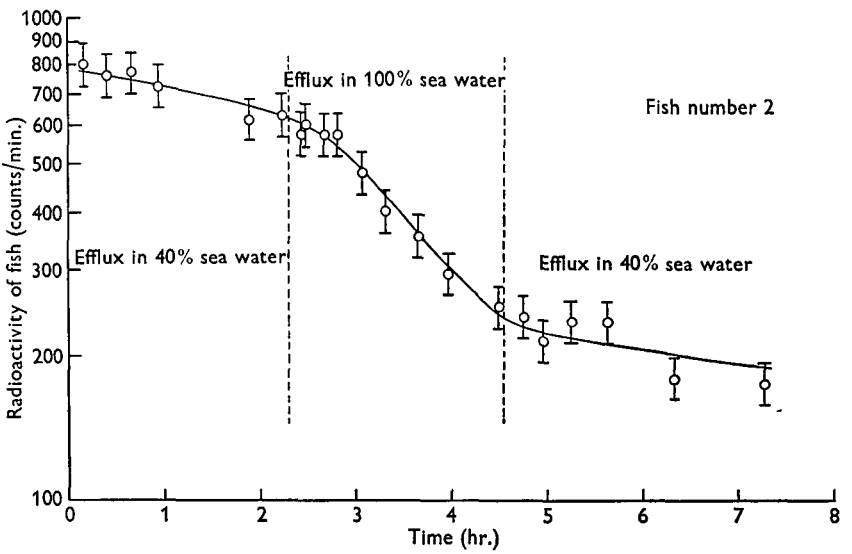

Fig. 4. The relation between the radioactivity of an animal loaded with ^{24}Na and the time after washing with non-radioactive media. The broken lines show when the washing medium was changed.

were similar. The efflux of sodium was also studied in 40 % s.w., followed by 150 % s.w. and then by 40 % s.w. (see Fig. 5).

The temperature-dependence of the efflux rate in 100 and 10 % s.w. was studied by perfusing with the medium previously cooled to 8° C. in a refrigerated water-bath.

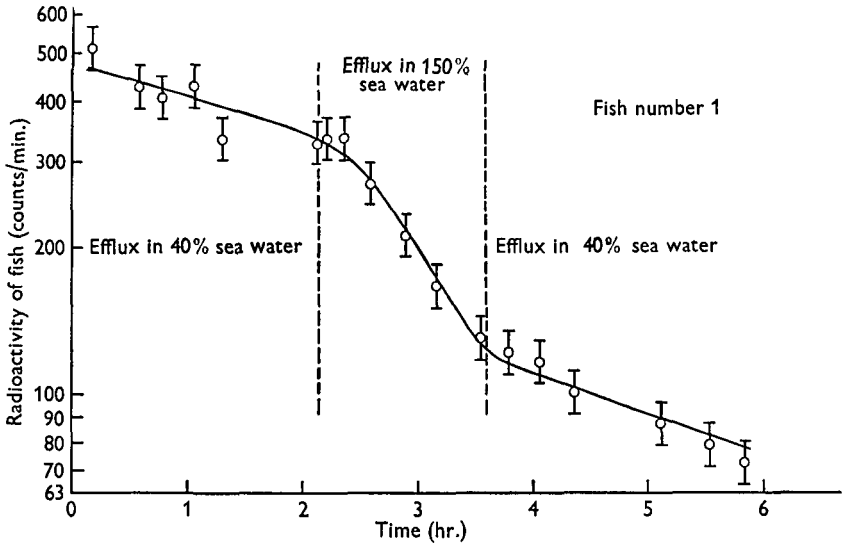

Fig. 5. The relation between the radioactivity of an animal loaded with ^{24}Na and the time after washing with non-radioactive media. The broken lines show when the washing medium was changed.

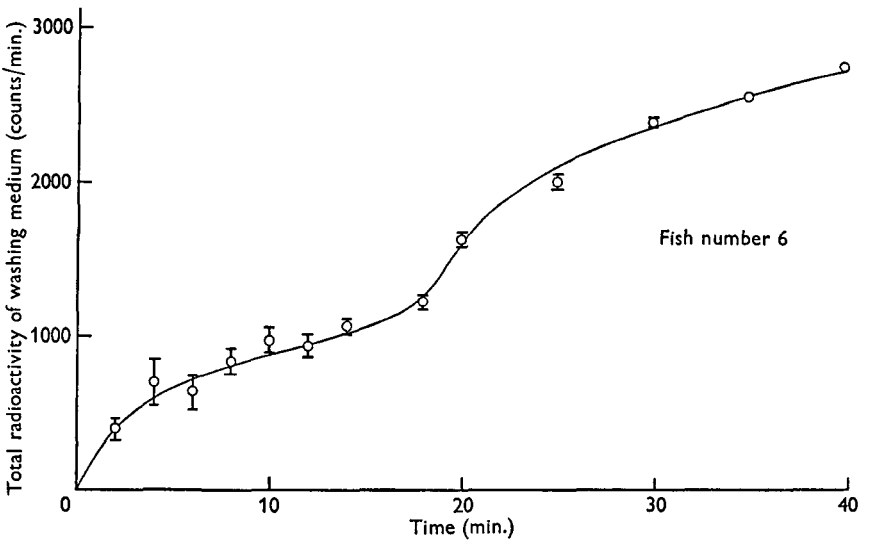

Fig. 6. The rise in radioactivity of the external medium (100 % s.w.) with time after an animal loaded with ^{24}Na had been transferred to it from 40 % s.w.

In order to measure more accurately the change of efflux rate when the medium was altered, other efflux experiments were carried out with a liquid G.M. tube. These were designed to show the rapid rise in activity of the washing medium of 100 % s.w. when an 'active' animal began to osmo-regulate after transference from 40 % s.w. The

advantage of this method was that estimates of the efflux of ^{24}Na could be made at small time intervals after transference from the almost iso-osmotic medium to the hyper-osmotic medium.

In the first of these experiments the animal was 'loaded' in an active 40 % S.W solution, removed and washed with inactive medium. It was transferred to a volum

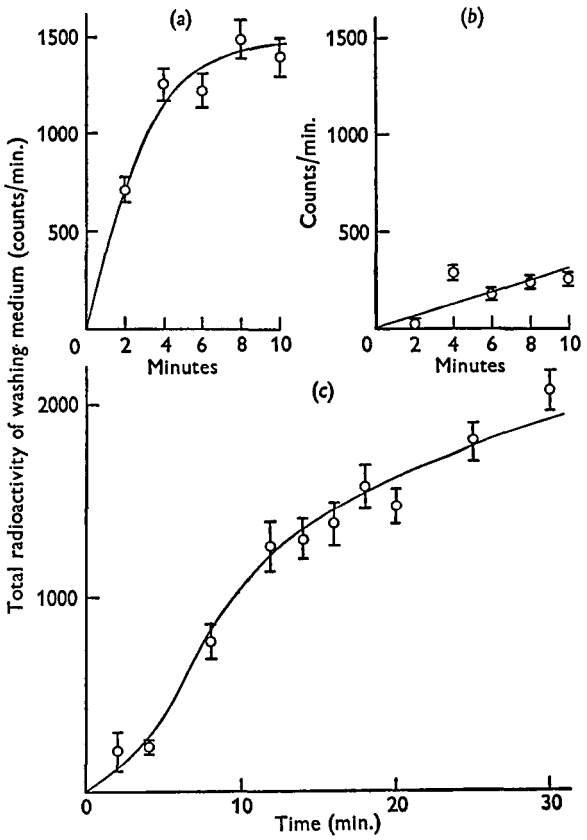

Fig. 7. The rise in radioactivity of various washing media with time after animals loaded with ^{24}Na had been transferred to them. In (*a*) and (*b*) the external medium was 40 % S.W. and in (*c*) 100 % S.W.

of 40 % S.W. for a certain time; it was then removed, washed with inactive medium and transferred to a known volume (200 ml.) of inactive 100 % S.W. continuously mixed. At various time intervals 10 ml. samples were pipetted from this washing solution and transferred to test-tubes. These samples were counted in the liquid G.M. tube under standard conditions, and corrections were made to the activities of the samples due to the removal of activity in the preceding samples. The result of this experiment is shown in Fig. 6. The initial sharp rise in the efflux followed by a levelling off was probably due to the loss of gut fluid or urine as a result of the stress of being handled. The efflux from 6 to 16 min. is probably characteristic of the flux in 40 % S.W. After 16 min. the efflux shown is approximately three times the efflux shown from 6 to 16 min., and this indicates that the animal is beginning to osmo-regulate a short time

after transfer to 100 % S.W. After about 30 min. the activity of the washing solution tends to a finite value because the source of the activity (i.e. the fish) is finite.

Because of the unusual result of the above experiment a new experiment was designed. The animal was 'loaded', removed, washed and transferred to inactive 40 % S.W. as before. Samples (10 ml.) were removed at various time intervals (see Fig. 7*a*) and then the animal was removed, washed and transferred to another 40 % S.W. solution of known volume. Again 10 ml. samples were removed (see Fig. 7*b*) as before; the animal was finally removed, washed and transferred to a known volume of 100 % S.W. as before. Again 10 ml. samples were removed (see Fig. 7*c*) at various time intervals and counted as above. Once again a sharp rise was obtained in the first 4 min., after which the efflux was almost constant (Fig. 7*a* 4–10 min.) and similar to the efflux in Fig. 7*b*.

Influx experiments

The influxes of sodium in 100 and 40 % S.W. have been studied in the steady state. Approximately 2 mc. were added to each volume (*ca.* 250 ml.) of the external media. At various time intervals samples of blood were obtained from the animals and their activities determined. Samples of the active external media were also taken simultaneously with the blood samples. The influxes in 100 % and 40 % S.W. have been determined by the two methods outlined in the theoretical discussion and are given in Fig. 3. These are considered to be less accurate than the efflux measurements because of the low counting rates and small magnitudes (*ca.* 1 mg.) of the blood samples.

Potential measurements

The results of the potential measurements are shown in Fig. 2. In all experiments the P.D. between the blood and the medium did not reach a steady value till several hours after insertion of the micro-electrode. Invariably the P.D. increased in magnitude almost linearly in the first few hours of measurement and then tended asymptotically to a final steady value. This general type of variation in P.D. might be caused by some electrical short-circuiting across the body surface between the electrodes. This short-circuiting would be expected to decrease as a function of time as the body surface between the point of insertion of the micro-electrode and the liquid surface progressively dried, and also it would be expected to be more effective in the more concentrated media because of the higher electrical conductivity. This effect was actually observed. In any measurement the final P.D. did not differ in magnitude from the initial P.D. by more than 30 mV. and on completion of the measurements the animals appeared normally active; therefore it was assumed that the P.D. had been measured while the gills were functioning normally. Generally, measurements were made at 20 min. intervals and the P.D. was judged to be at a steady value if it did not change by more than 1 mV. in 1 hr. During the course of the experiments small movements of the animal disturbed the value of the P.D. but a suitable return was attained within several minutes. The ionic (sodium, potassium and chloride) concentrations in the blood were always measured on the conclusion of these experiments and thus estimates of the Nernst potentials, $E_j = RT/z_jF \ln C_o/C_i$, for the three ions were obtained. In addition to the membrane potential E, a knowledge of E_j for the individual ions j was required before any attempt could be made to determine whether active pumping mechanisms occur in certain physiological conditions.

DISCUSSION

The main results of the flux experiments are that the effluxes of sodium in 10 and 40 % S.W. are similar while the efflux is much faster in 100 % S.W.; in addition the change of efflux when the animal is transferred from 40 to 100 % S.W. is rapid.

From the data of Croghan (1958) on *Artemia salina* it was considered that both the sodium and chloride in the extracellular space underwent rapid exchange diffusion with the sodium and chloride in the medium. Considering the extreme limiting case that the total sodium efflux (20 mM. Na/l. blood/hr.) in 10 and 40 % S.W. is an exchange-diffusion flux across the gills then the exchange-diffusion flux in 100 % S.W. must be similar because exchange diffusion is independent of concentration of the ion (Ussing, 1952). Because the total efflux across the gills in 100 % S.W. is 100 mM. Na/l. blood/hr. then there must be an additional component of efflux (80 mM. Na/l. blood/hr.) to the exchange flux. This latter component of sodium efflux would be presumably an active efflux which balances the sodium uptake in the animal's gut. It is interesting to note that later an active efflux of sodium (also 80 mM. Na/l. blood/hr.) will be calculated assuming that there is no exchange diffusion in the gill membranes. There is some additional evidence for postulating an active sodium efflux in 100 % S.W. The Q_{10} for the efflux in 100 % S.W. is approximately 3 while in 10 % S.W. it is approximately 1. It is acknowledged that the Q_{10} value is not a valuable criterion for active transport of ions, but in this case of identical experiments in different external media the discrepancy in Q_{10} values would indicate that there are different mechanisms involved in sodium efflux in 100 and 10 % S.W.

The simplest explanation of these efflux measurements on the blenny is that in 10 and 40 % S.W. the sodium efflux is passive and in 100 % S.W. there is an active component of sodium efflux.

In hyper-osmotic media it has been assumed that the animal prevents dehydration by swallowing sea water and reducing urine excretion to a negligible quantity. Thus in 40 % and particularly 100 % S.W. the channels for salt and water transport are almost exclusively the gut and the gills. In 100 % S.W. the efflux of sodium across the gills is balanced by the influx, J_{in}, across the gills plus the influx of sodium across the gut when the animal is in a steady state. The efflux across the gills in 100 % S.W. may be purely passive, J_{out}, or composed of a passive efflux J_{out} plus an active transport component. In hypo-osmotic media it has been assumed that the animal prevents swelling by excreting a very hypotonic urine. Thus in 10 % S.W. the channels for salt transport are the gills while the channels for water transport are mainly the gills and the kidneys. In 10 % S.W. the passive efflux, J_{out}, of sodium across the gills might be balanced by a purely passive influx J_{in} across the gills or a passive influx J_{in} plus an active influx.

Sodium and chloride 'pumps'

In Table 1 the values of the Nernst potentials for sodium and chloride are given along with the P.D. (E) measurements across the gill membranes. In measuring E the assumption was made that the lowest resistance pathway between the electrodes would be across such a permeable structure as the gill membranes. There are likely to be relatively larger resistance pathways associated with the gut and kidneys.

If the ions are moving passively under the action of the purely physical forces of chemical and electrical potential gradients then E will be equal in magnitude to the Nernst potential for the ions. In 100 and 150% S.W. it is difficult to decide on this basis whether sodium is moving in passive equilibrium or being actively pumped. In 10 and 40% S.W., however, it appears that the sodium is not in passive equilibrium and the data suggest an active inward pumping of sodium across the gills. It also appears from Table 1 that chloride is not in passive equilibrium in all of the media used in these experiments. In 40, 100 and 150% S.W. it appears that chloride is actively pumped out from the animal while in 10% S.W. there is evidence of an inwardly directed pumping of chloride ions.

Table 1. *Sodium and chloride Nernst potentials and gill membrane* P.D.

Medium (% S.W.)	$E_{Na} = \frac{RT}{zF} \ln \frac{[Na_o]}{[Na_i]}$ Mean ± S.E. (mV.)	$E_{Cl} = \frac{RT}{zF} \ln \frac{[Cl_o]}{[Cl_i]}$ Mean ± S.E. (mV.)	E Mean ± S.E. (mV.)
10	−18 ± 3	+16 ± 3	− 3 ± 2
40	+ 4 ± 2	−11 ± 2	+23 ± 2
100	+26 ± 2	−29 ± 2	+23 ± 1
150	+36 ± 2	−38 ± 2	+29·5 ± 3·5

The most straightforward explanation of these data is that in the hyper-osmotic media (i.e. 40, 100 and 150% S.W.) the chloride is actively pumped out across the gills, while in 10% S.W. the 'chloride pump' changes direction and there is also an active inward pumping of sodium in 40 and 10% S.W.

The role of potassium is difficult to assess because of the inaccuracy in the measurement of serum concentrations; in all media the serum concentrations were found to be larger than 10 mM./l. whereas the data given by Lockwood (1961) on the blood of marine teleosts shows values between 2 and 8 mM./l. for potassium. If a more likely constant value of 5 mM./l. is assumed, this would suggest that potassium might be in passive equilibrium in 100% S.W. and actively pumped inwards in 10 and 40% S.W.

While the animal is in 10 and 40% S.W. it may therefore be assumed that any active pumping of sodium is inwards across the gills. In addition, assuming there is no exchange diffusion, it is found that the ratio of the passive efflux, J_{out}, in 40% S.W. to the passive efflux in 10% S.W. is approximately equal to the ratio of the experimental values for efflux when E is taken as +23 mV. (inside positive) in 40% S.W. and as 0 mV. in 10% S.W. Both of these P.Ds lie within the experimental range of values, and if it is now assumed that the Goldman theory fits the experimental data then a calculation of the passive efflux in 100% S.W. is possible from the ratio of passive efflux in 100% to the passive efflux in 40% S.W., which is known.

Calculation of passive efflux of sodium in 100% *sea water*

We have,

$$\frac{\text{passive efflux in 100\% S.W.}}{\text{passive efflux in 40\% S.W.}} = \frac{\left[\frac{FE/RT}{1-\exp(FE/RT)}[Na_i]\exp(FE/RT)\right]_{100\%\,S.W.}}{\left[\frac{FE/RT}{1-\exp(FE/RT)}[Na_i]\exp(FE/RT)\right]_{40\%\,S.W.}}.$$

For the blenny in 100 % S.W.,

$$E = +23 \text{ mV.}$$

$$[\text{Na}_i] = 170 \text{ mM./l.} = 1{\cdot}7 \times 10^{-4} \text{ moles cm.}^{-3}.$$

For the blenny in 40 % S.W.,

$$E = +23 \text{ mV.}$$

$$[\text{Na}_i] = 160 \text{ mM./l.} = 1{\cdot}6 \times 10^{-4} \text{ moles cm.}^{-3}.$$

Hence,

$$\text{Passive efflux in 100 \% S.W.} = (1{\cdot}06) \times \text{passive efflux in 40 \% S.W.},$$

i.e. passive efflux in 100 % S.W. is 21 mM. Na/l. blood/hr. if the passive efflux in 40 % S.W. is taken as 20 mM. Na/l. blood/hr.

Although the P.D. measurements in 100 % S.W. were inconclusive in determining if active sodium transport occurred across the gills some conclusions may be derived from the fluxes. The passive efflux J_{out} has been calculated for 100 % S.W. and this is not found equal to the experimental value of the total efflux. It may be concluded that the total efflux of sodium has a passive component, J_{out}, and an active component, J_{act}.

It is now possible to calculate an active transport rate of sodium in 100 % S.W.

Calculation of active transport rate of sodium in 100 % *sea water*

In the following calculation it is assumed that exchange diffusion does not occur in the gill membranes and that the animal in 100 % S.W. is swallowing sea water and absorbing a sodium chloride solution (isotonic with 100 % S.W.) from its gut while it actively excretes sodium through the gills. Because the total influx of sodium must equal the total efflux from the animal in a steady state, then the swallowing rate, J_{sw}, is given by

$$J_{\text{in}} + J_{\text{sw}} = J_{\text{out}} + J_{\text{act}},$$

where J_{in}, J_{out} and J_{act} have been described before. For the blenny in 100 % S.W.,

$$\frac{J_{\text{in}}}{J_{\text{out}}} = \frac{[\text{Na}_o]}{[\text{Na}_i] \exp (FE/RT)},$$

where

$$[\text{Na}_o] = 470 \text{ mM./l.} = 4{\cdot}7 \times 10^{-4} \text{ moles cm.}^{-3},$$

$$[\text{Na}_i] = 170 \text{ mM./l.} = 1{\cdot}7 \times 10^{-4} \text{ moles cm.}^{-3},$$

$$E = +23 \text{ mV.}$$

Hence

$$\frac{J_{\text{in}}}{J_{\text{out}}} \simeq 1.$$

Therefore

$$\begin{aligned} J_{\text{sw}} &\simeq J_{\text{act}} \\ &= \text{total efflux} - J_{\text{out}} \\ &= 79 \text{ mM. Na/l. blood/hr.} \end{aligned}$$

But,

$$\text{water content} = 80 \text{ \% wet weight},$$

$$\text{sodium space} = 45 \text{ \% water content.}$$

Hence,

$$J_{sw} = 0{\cdot}8 \times 0{\cdot}45 \times 79 \text{ mM. Na/kg. fish/hr.}$$
$$= 27 \text{ mM. Na/kg. fish/hr.}$$
$$= \frac{27 \times 100}{[Na_o]} \% \text{ body weight/hr.}$$
$$= 5{\cdot}7 \% \text{ body weight/hr.}$$

Thus in 100 % S.W. the swallowing rate of sodium is 27 mM. Na/kg. fish/hr. or 6 % body weight/hr. This active transport rate is comparable with the data of Mullins (1950) on the stickleback (4 % body weight/hr.) and of Smith (1930) on the eel, *Anguilla* (1 % body weight/hr.). However, there are several objections to the use of the Goldman theory in this system. The foremost objection is that there are likely to be at least two biological membranes in the fish gills and the P.D. existing across this system would be a complicated function of ionic concentrations in all three compartments separated by these two membranes.

Calculations of J_{in}/J_{out} for sodium and chloride ions in 100 and 10 % S.W. also indicate that there is an active chloride efflux in 100 % S.W. and that there are active sodium and chloride influxes in 10 % S.W.

Osmo-regulatory adaptation in 100 % *sea water*

An interesting point which arises in Figs. 4 and 5 is the rapidity with which the osmo-regulatory mechanism begins to operate. In Fig. 4 it appears that the animal actively excretes sodium shortly after its transference from 40 to 100 % S.W. Houston (1959) observed that in the adaptation of the steelhead trout to sea water there were adjustive and regulative phases in the response. He found that during the initial adjustive phase the animal must endure increased electrolyte levels in the plasma. The length of the adjustive phase (between 80 and 170 hr.) was of a similar magnitude to those obtained by Black (1951) on chum salmon fry (36 hr.), and by Keys (1933) on the eel, *Anguilla vulgaris* (approximately 50 hr.). Parry (1960) studied the survival and osmotic regulation of young salmonid fishes following transfer from fresh water to various dilutions of sea water. From the data of the three species of salmon which were used, it was shown that, provided animals could tolerate the increase in the plasma electrolyte levels in 100 % S.W., they required about 200 hr. to return to the normal blood concentration.

The experiments with the liquid G.M. tube, which were designed to show up the rapidity of the adjustive phase in the blenny, are shown in Figs. 6 and 7. In Fig. 6 the initial sharp rise in the efflux of sodium is very puzzling. It could be explained on the basis that the fish was releasing a large quantity of urine (and/or gut fluid) in response to the stress of being transferred from one washing medium to another. Fig. 7 shows the results of an experiment designed to study both the effect on the efflux of transferring the animal from one medium to another and also the rapidity with which osmo-regulation acts. Fig. 7*a* tends to support the hypothesis that handling may stimulate a sudden release of urine, for the animal experienced no change in the concentration of its external medium. There is no such rapid efflux of sodium, however, in Fig. 7*b*, which exhibits the efflux in a similar almost iso-osmotic medium. In

Fig. 7*c* the animal appears to be osmo-regulating almost immediately (at least within 5 min.) after being placed in the 100 % S.W., and this gives support to the evidence that the blenny has a very short adjustive phase when transferred to 100 % S.W. from 40 % S.W. This rapid adaptive ability in osmo-regulation in 100 % S.W. conforms with the possible sudden variations in the external concentration which this animal may undergo, for it is a small littoral fish which extends into brackish water.

SUMMARY

1. The sodium effluxes between the blood and the medium have been studied in *Blennius pholis* in 10, 40 and 100 % S.W. and in transfer from 40 to 100 % S.W. under equilibrium conditions with the isotope ^{24}Na. In addition the sodium influxes have been studied in 40 and 100 % S.W. in a similar manner.

2. The total flux in 100 % S.W. has been found to be 100 mM. Na/l. blood/hr., and in 40 and 10 % S.W. it has been found to be 20 mM. Na/l. blood/hr.

3. The results are interpreted as showing that the presence or absence of exchange diffusion does not alter the estimation of the active sodium efflux across the gills in 100 % S.W.

4. The efflux experiments showed that the animal had a rapid adaptive ability to osmo-regulate upon transference from 40 to 100 % S.W.

5. The electric potential differences between the blood and external medium have been measured in animals adapted to 10, 40, 100 and 150 % S.W. and the blood serum concentrations of sodium and chloride ions have been measured in these media.

6. The mean potential differences ± standard deviation in 10, 40, 100 and 150 % S.W. have been found to be $-3 \pm 4{\cdot}5$ mV., $+23 \pm 4$ mV., $+23 \pm 3$ mV. and $+29{\cdot}5 \pm 5$ mV. respectively (external medium taken as reference).

7. The results are interpreted as showing that there is active outward excretion of chloride ions in animals adapted to 150, 100 and 40 % S.W. and active inward absorption of chloride ions in 10 % S.W. and of sodium ions in animals adapted to 40 and 10 % S.W.

8. The drinking rate in 100 % S.W. has been calculated to be 27 mM. Na/kg. fish/hr. or 6 % body weight/hr.

I wish to thank Dr W. T. W. Potts and Dr P. C. Croghan for discussion, interest and valuable advice. I also wish to thank the Department of Scientific and Industrial Research for a maintenance grant.

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