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ION AND WATER TRANSPORT IN ISOLATED INTESTINE OF THE MARINE TELEOST, *COTTUS SCORPIUS*

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INTRODUCTION

Homer Smith (1930) originally outlined the special osmo-regulatory mechanisms of the marine teleosts; he postulated that these animals continually drink sea water, absorb monovalent ions in the gut and simultaneously excrete excess ions, mainly through the gills, in order to maintain a constant blood concentration lower than that of the external sea water. There have been no reported *in vitro* transport studies, however, of the epithelial membranes involved in the regulation processes although several workers (Keys, 1933; Mullins, 1950; House, 1963) have performed *in vivo* ion transport experiments which confirmed Smith's view of osmotic regulation. These experiments have offered unfortunately no description of the nature of the osmo-regulatory processes occurring in the gills, kidneys and, in particular, the intestine. Many workers have investigated ion and water movements in the isolated intestines of such vertebrates as the rat, rabbit and dog, but it is not completely satisfactory to extrapolate from their results concerning the fine mechanisms of ion and water transport to conclusions applying to marine teleosts.

The purpose of the investigation reported here was to determine the nature of ion and water transport across the small intestine of the marine teleost, *Cottus scorpius*. Some of these results have previously been briefly reported elsewhere (House & Green, 1963).

MATERIALS AND METHODS

The animals were obtained from Pittenweem, Fife, and were kept in a marine aquarium until used. All experiments were performed at ambient room temperature (*c.* 18° C.).

Each animal was killed by a sharp blow on the head and after opening the abdomen the small intestine was excised. The excised segment was immersed in a beaker containing the experimental medium; after a few minutes the segment was transferred to a new volume of the medium in a dish containing an immersed fixed Perspex rod. The intestinal segment was usually subdivided into three portions at this stage. The tissue was gently pulled over the rod to remove remaining intestinal contents and the adhering connective tissue was cut away.

Table 1 shows the composition of the experimental solutions used in this study. Salines A, B and C will be referred to as Ringer, sulphate Ringer and choline Ringer

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respectively; saline E was an artificial sea water modified from Hale (1958). The pH of salines A, B, C and D was 7·2.

In all of the experiments the tissue was equilibrated with the experimental medium, in which it was to be bathed initially, for at least 30 min. before commencing measurements.

Table 1. *Composition of experimental solutions* (mM./l.)

	A	B	C	D	E
NaCl	205·0	—	—	205·0	410·6
KCl	8·0	8·0	8·0	8·0	10·0
$CaCl_2$	1·6	1·6	1·6	1·6	10·2
$MgCl_2$	1·0	1·0	1·0	1·0	53·6
$NaHCO_3$	2·3	2·3	2·3	2·3	2·3
KH_2PO_4	0·5	0·5	0·5	0·5	—
Na_2SO_4	—	102·5	—	—	28·2
Choline Cl	—	—	205·0	—	—
Glucose	2·8	2·8	2·8	2·8	—
Sucrose	—	50·0	—	50·0	—

Net water transport was measured by the change in weight of normal (i.e. non-everted) sacs, filled with the experimental saline, during half-hour incubation periods in the serosal medium. In a few preliminary experiments (House & Green, 1963) net water flux was measured by a gravimetric chamber technique to be described elsewhere (House, 1964). This latter technique also permitted measurement of the electrical potential difference (p.d.) across the intestine; both methods of measuring water flux gave results in agreement but the sac technique was preferred because it allowed simultaneous observations on numerous pieces of tissue. The sacs were weighed once only at the end of each experimental period after draining them three times on the side of a dry beaker going progressively around the circumference of the beaker so that all sides of the sac would be drained. In this measurement the accuracy is difficult to assess because of two factors: (i) inconsistencies in the draining operation, and (ii) changes in the weight of the tissue itself, which might be wholly or partially responsible for the observed weight change. In a preliminary study it was found after four rapid successive weighings of a typical normal filled sac that its weight could be expressed to an accuracy of about $\pm$ 2 mg. During the half-hour incubation periods in Ringer a typical normal sac, filled with Ringer, lost about 10 mg. and, moreover, the loss was found to be approximately the same over several successive experimental periods. It seems safe, therefore, to consider that the weight changes in normal filled sacs result from net flows of water across the wall of the intestine. House (1964), performing analogous experiments on isolated frog skin, found that tissue-weight changes were small and did not interfere with the measurement of net water fluxes as low as 0·5 μl. cm.$^{-2}$ hr.$^{-1}$, and we conclude that it would be most unlikely that the observed consistent 'net flows of water' resulted from tissue-weight change only.

The equations governing the exchange of radioactive ions between two compartments have been outlined by Ussing (1949) and others. Consider the following case applicable to the present experiments.

If a normal filled sac containing no radioactive ions is placed in contact with a large volume of serosal solution in which the total concentration (μequiv. cm.$^{-3}$) of the

ion species under study is C_s and the serosal concentration of labelled ions is C_s^* (counting rate cm.$^{-3}$), then the initial rate of entry of labelled ions is

$$\frac{dC_m^*}{dt} = J_{sm}\frac{AC_s^*}{vC_s}, \tag{1}$$

where A is the surface area (cm.^{2}) across which ion exchange takes place, v is the volume of the mucosal solution (cm.^{3}), C_m^* is the mucosal concentration of labelled ions and J_{sm} is the serosal to mucosal flux (μequiv. cm.$^{-2}$ hr.$^{-1}$) of the ion. After some time, when the radioactive ions are crossing the surface in both directions, the rate of change of mucosal radioactivity is

$$\frac{dC_m^*}{dt} = J_{sm}\frac{AC_s^*}{vC_s} - J_{ms}\frac{AC_m^*}{vC_m}, \tag{2}$$

where C_m is the mucosal concentration of the ion species and J_{ms} is the mucosal to serosal flux of the ion. In all of the isotopic experiments identical solutions bathed both sides of the tissue, i.e. $C_m = C_s$.

In a washout experiment, in which the radioactive ion is not allowed to accumulate in the serosal medium, C_s^* may be neglected and the solution of equation (2) is

$$C_m^* = [C_m^*]_{(t=0)}\exp\left\{-\frac{J_{ms}At)}{vC_m}\right\}, \tag{3}$$

where $[C_m^*]_{(t=0)}$ is the initial concentration of labelled ion in the mucosal solution. Provided v and A are known then values of J_{sm} and J_{ms} may be obtained from uptake and washout experiments on the same sacs by equations (1) and (3).

Unidirectional sodium fluxes, from serosa to mucosa and mucosa to serosa, were determined on the same individual sacs using ^{22}Na. The serosal to mucosal sodium flux, J_{sm}, was found by immersing normal filled sacs in a continuously aerated saline loaded with ^{22}Na (volume $\doteq$ 250 ml.; specific activity $\doteq$ 0·03 μc./ml.) and monitoring the sacs in a well-type scintillation counter at known times after immersion. Before the counting procedure the sacs were washed by four rapid successive immersions in three separate volumes of the experimental saline. This washing lasted less than 20 sec. and its efficiency was found by placing two normal filled sacs in radioactive Ringer for 60 sec. and then monitoring the sacs after the same washing. The specific activity of the sacs was about 1 % of the specific activity of the radioactive Ringer. Values of the sodium flux, J_{sm}, were calculated from equation (1) after making allowance for the uptake of the isotope into the extracellular and intracellular spaces of the tissue. The sacs were then transferred to a large volume (*c.* 250 ml.) of aerated saline and the mucosal to serosal fluxes of sodium were determined from the decrease in specific activity of the sacs with time according to equation (3).

Measurements of the unidirectional chloride fluxes were made with ^{36}Cl (specific activity of radioactive saline $\doteq$ 0·02 μc./ml.) and the apparatus shown in Fig. 1 *a* was employed. Segments of small intestine were tied with cotton thread on to the Perspex cylinder as shown, with the other open end of the segment tied on to a grooved Perspex plug, and the mucosal volume was filled by syringe via the screw hole in the top of the chamber. After a known amount of saline had been introduced the apparatus was immersed in the radioactive serosal medium. Samples (*c.* 100 μl.) were removed by micropipettes from the mucosal solution at known times and these were

transferred to weighed planchets. The weights of the radioactive samples were recorded and the planchets were dried on a hot plate at about 60° C. and then counted under an end-window Geiger counter. Values of J_{sm} were calculated by equation (1) after a correction had been applied for the reduction in mucosal volume, v, due to the removal of samples for counting. Because of the occasional significant reduction in mucosal volume (maximum reduction about 25% of original volume) the mucosal to serosal chloride fluxes were determined with the same apparatus on different pieces of tissue by introducing a known volume of radioactive saline into the mucosal compartment and then placing the chambers in non-radioactive saline. Values of J_{ms} were then calculated from equation (3) after observing the decline in C_m^* with time. Throughout the measurements of chloride flux the hole in the top of the Perspex chamber was closed by a Nylon screw to minimize evaporation loss and this was removed prior to each sampling.

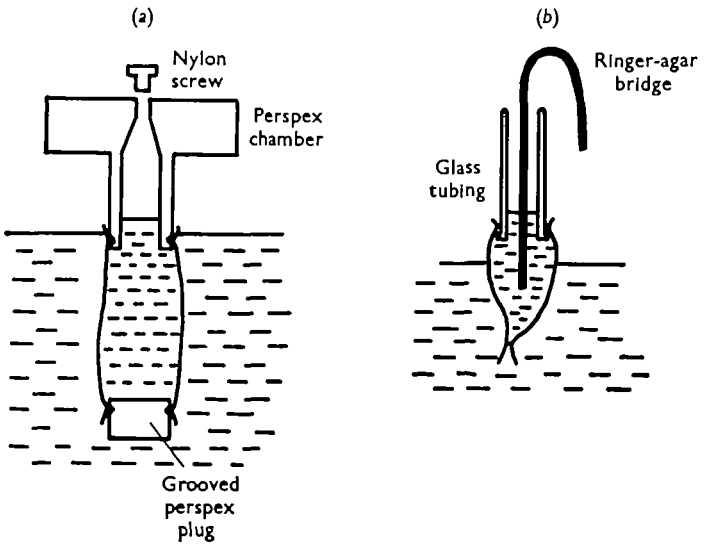

Fig. 1. (*a*) The apparatus for the measurement of unidirectional chloride fluxes across intestinal segments. (*b*) The apparatus for the measurement of electric potential differences between the serosal and mucosal media. In (*a*) and (*b*) the tissue segments were non-everted.

In all of the experiments on fluxes of water and ions the area of tissue was measured at the end of the experiment by cutting along a line parallel to the axis of the cylindrical gut surface and spreading the tissue on filter paper. The area was estimated by measuring two adjacent sides of the approximately rectangular surface obtained and the maximum error in the apparent surface area was less than 10%.

Measurements of the potential difference (p.d.) across the wall of the intestine bathed by various media were made with Ringer-agar bridges placed in the mucosal and serosal media as shown in Fig. 1*b*. The p.d. between these bridges was measured by a pair of calomel electrodes leading into a high impedance null-reading millivoltmeter (pH Meter 4, Radiometer, Copenhagen). The junction potentials between calomel electrode and bridge were measured in the experimental media prior to measuring the p.d. across the intestinal wall (the transepithelial p.d.). Potential

measurements were performed at half-hour intervals over at least 2 hr. and the p.d. was measured to $\pm 0{\cdot}5$ millivolt (mV.). The sign convention chosen in this paper for the statement of p.d.'s is that the potential of the serosal medium has always been measured with the mucosal medium as reference.

To check whether or not the various experimental media used in these studies were harmful to the tissue, the respiration rate was measured in these media. Respiration was followed in a Warburg apparatus and the results of these observations were expressed in terms of μl. of oxygen consumed per mg. dry weight of tissue per hour at 25° C.

RESULTS

Table 2 shows the results of measurements of the p.d. existing across the small intestine of *Cottus* when it is bathed on both surfaces by identical solutions. In all cases the p.d. is not appreciably different from zero and this is in general agreement with previous *in vitro* measurements on the small intestine of other animals. Ussing, Kruhøffer, Hess Thaysen & Thorn (1960) have commented on the fact that the small intestines of some vertebrates maintain p.d.'s of only a few mV. and they consider that this arises because of the large permeability to ions of this part of the intestinal tract. Curran & Solomon (1957), however, found that the active transport potentials of sodium and chloride ions in the rat ileum nearly neutralize each other, a condition which also leads to a low measured p.d.

Table 2. *Transepithelial potential difference and respiration rate in various media*

Medium	Potential difference (mV.) (mean ± S.E.)	Respiration rate at 25° C (μl. O_2/mg dry wt. hr.) (mean ± S.E.)
Ringer	$+0{\cdot}6 \pm 0{\cdot}3$ (12 sacs)	$0{\cdot}4 \pm 0{\cdot}2$ (34, 7)*
Sulphate Ringer	$+2{\cdot}2 \pm 0{\cdot}2$ (6 sacs)	$0{\cdot}3 \pm 0{\cdot}2$ (16, 5)
Choline Ringer	$+0{\cdot}8 \pm 0\,3$ (6 sacs)	$0{\cdot}3 \pm 0{\cdot}2$ (10, 3)
Artificial sea water	$+1{\cdot}3 \pm 0{\cdot}2$ (4 sacs)	$0{\cdot}1 \pm 0{\cdot}2$ (6, 2)

* The first number in each bracket denotes the number of hourly measurements, while the second denotes the number of pieces of intestine studied.

Table 2 also shows that in all of these experimental conditions the tissue remains alive; on each piece of tissue the oxygen consumption was measured at hourly intervals over at least 3 hr. and the respiration rate was approximately constant over this period. In all cases the tissue consumes oxygen at similar rates except, perhaps, when bathed by artificial sea water; it is not possible to conclude from the few measurements in this medium, however, whether or not metabolism has been significantly depressed. These data provide an adequate foundation for the design of flux experiments to determine the nature of sodium and chloride movements across the intestine.

Ion fluxes

Since the observed p.d. across the small intestine was not significantly different from zero it was considered unnecessary to measure ion fluxes in an electrically 'short-circuited' condition and it was assumed that there was no appreciable electrical driving force on the ions during the experiments with isotopes.

The measurement of serosal to mucosal and mucosal to serosal fluxes of sodium was achieved by the 'whole-body' counting of normal sacs labelled with ^{22}Na. This technique permitted the measurement of J_{ms} and J_{sm} on the same sac after allowance had been made for ^{22}Na present in the tissue itself (i.e. extracellular and intracellular spaces). Fig. 2 shows the result of an uptake and washout experiment on a typical sac filled with Ringer and placed in Ringer loaded with ^{22}Na. The simplest and most plausible explanation of the uptake curve is that the initial steep rise (0–30 min.) in the radioactivity of the sac is due to the increase in labelled sodium in the tissue and thereafter the slower rise (30–60 min.) gives the steady state flux of sodium into the mucosal solution. Thus the slope of X can be used in the calculation of J_{sm} by equation (1). The washout curve does not give such a sharp demarcation between the two slopes, presumably because the rate at which sodium leaves the mucosal solution is approximately equal to the initial rate of loss from the tissue space. Curve Y permits a calculation of J_{ms} by equation (3). In this way values of J_{sm} and J_{ms} were obtained for sacs bathed on both sides by Ringer and by sulphate Ringer and these results are expressed in Table 3.

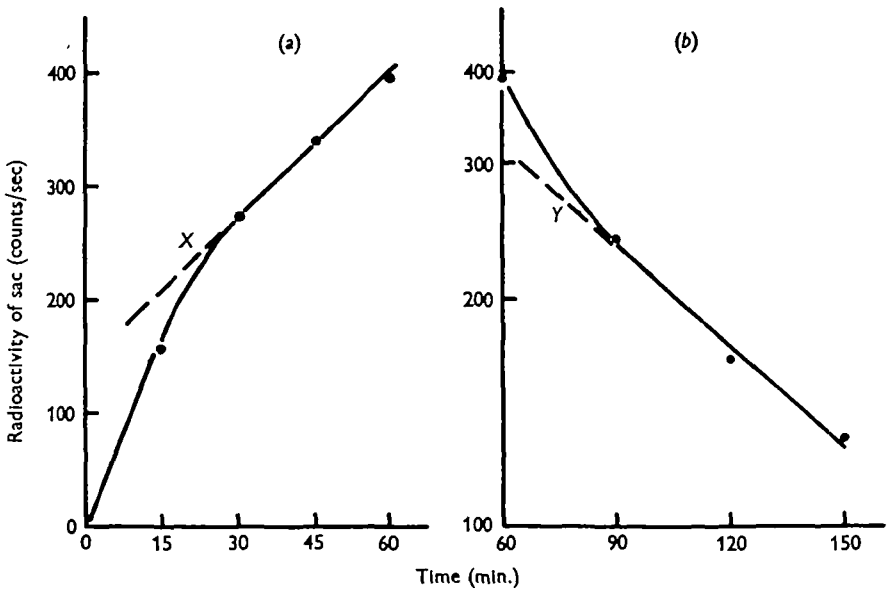

Fig. 2. (*a*) The rise in radioactivity of a typical normal sac, filled with Ringer, after immersion in ^{22}Na-loaded Ringer. (*b*) The decline in the radioactivity of the same sac after transference to non-radioactive Ringer.

These observations prove that there is a net transfer of sodium ions from the mucosal to the serosal side of the small intestine when Ringer is present. These data also suggest a linkage between the net sodium flux and the presence of chloride in the bathing media since in our sulphate Ringer, which contained only a concentration of 13·2 mM./l. chloride, an appreciable reduction in net sodium flow occurs.

The average mucosal to serosal and serosal to mucosal fluxes of chloride are given in Table 4. It was found in these experiments, as in the sodium flux measurements, that after about 30 min. a steady-state flux of isotope was established and this agrees

with the observations of Schultz & Zalusky (1964) on the sodium fluxes across isolated rabbit ileum.

These results show a net transfer of chloride ions between identical Ringers and the magnitude of this net flux is similar to that of sodium in this medium (cf. Table 3). When the intestine is bathed by choline Ringer, however, net chloride transport is absent and this reinforces the view suggested by the sodium flux experiments that the flows of sodium and chloride ions are intimately coupled.

Table 3. *Unidirectional sodium fluxes between identical media*

	Sodium fluxes		
Medium	J_{sm}	J_{ms} (μequiv. cm.$^{-2}$ hr.$^{-1}$)	net $J_{ms} = J_{ms} - J_{sm}$
Ringer	8·4 ± 0·6 (13)	23·9 ± 0·7 (13)	12·9 ± 0·6 (13)
Sulphate Ringer	4·5 ± 0·3 (10)	6·8 ± 0·6 (10)	2·3 ± 0·5* (10)

Each result is quoted as mean ± S.E. (number of sacs).
* This value is significantly different from zero ($t = 4·4$, $P \simeq 0·005$).

Table 4. *Unidirectional chloride fluxes between identical media*

	Chloride fluxes	
Medium	J_{sm} (μequiv. cm.$^{-2}$ hr.$^{-1}$)	J_{ms}
Ringer	10·5 ± 0·9 (8)	21·9 ± 0·9 (9)
Choline Ringer	5·1 ± 1·0 (8)	5·4 ± 0·9 (8)

Each result is quoted as mean ± S.E. (number of sacs).

Osmotic water flux

It is widely held that the marine teleost absorbs water from swallowed sea water after dilution of this medium in the stomach and later in the intestine. The common view is that an osmotic flux of water from blood to lumen rapidly brings the mucosal solution to isotonicity with the blood and then water is absorbed by some mechanism dependent on the uptake of salt into the blood. Apparently no simple test of this hypothesis of water movement has been performed on an *in vitro* preparation of marine teleost gut.

In order to study the influence of osmotic water transfer in the process of water uptake from ingested sea water an estimate of the osmotic permeability (or hydraulic conductivity, L_p) of the small intestine of *Cottus* was obtained. L_p may be defined as the change in water flux produced by a change in osmotic gradient and this parameter was measured by creating an osmotic gradient across the intestine, which initially separated two isotonic solutions, under two different conditions: (1) initially both solutions were Ringers and there was a spontaneous mucosal to serosal net water flux which was altered when the serosal medium was replaced by saline D (containing 50 mM./l. sucrose); (2) initially both solutions were salines D and again there was a mucosal to serosal net flux of water altered by replacing the serosal medium by Ringer. In these experiments sucrose was assumed to be completely impermeant; if this is incorrect then the actual value for L_p will be larger than our estimate. The

osmotic permeability was found to be similar under both conditions and the mean ± S.E. value of L_p for 8 pieces of intestine was 0·22 ± 0·05 μl. water/cm.^{2} m-osmolar. hr. (or 2·4 × 10^{-6} cm. sec.$^{-1}$ atm.$^{-1}$). This value is close to that of the osmotic permeability of the luminal surface of the mucosal cells in the isolated jejunum of the rat (Lindeman & Solomon, 1962). On this evidence the marine teleost gut seems appropriately able to produce a rapid dilution of luminal sea water.

To investigate thoroughly the validity of the proposed mechanism of water uptake, several normal sacs were prepared with artificial sea water on both sides and after an equilibration period (30 min.) these sacs were transferred to an aerated Ringer solution. At certain times after transference the sacs were weighed and Fig. 3 shows the result of this experiment. These data clearly show that the smaller the luminal volume to be diluted the more rapidly will net water absorption take place after sea water enters the intestine and that osmotic water flow initially takes place from the blood into the lumen.

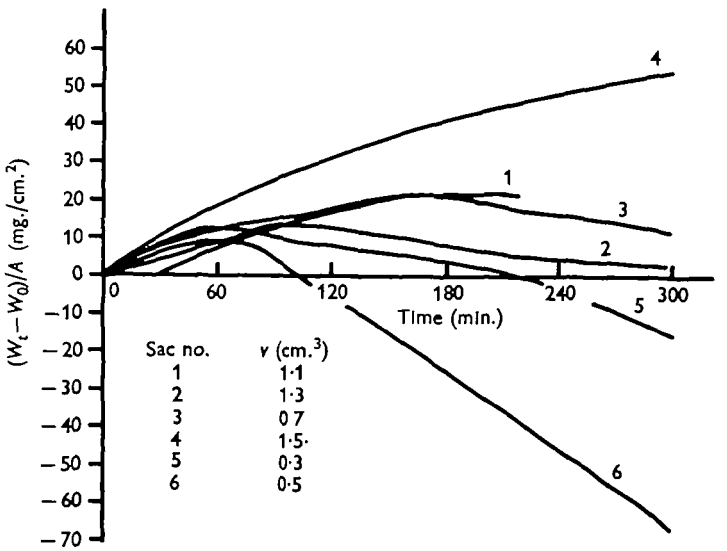

Fig 3. The time course of weight changes in normal sacs, filled initially with artificial sea water, when placed in Ringer. W_0 = initial weight of sac, W_t = weight of sac at time t and A = surface area of sac. In the inserted table v is the approximate volume of the mucosal solution in a sac and the number of each sac has been given on the corresponding curve.

Non-osmotic water flux

The experimental evidence described so far tends to support the view that the intestine can absorb water after dilution of the luminal contents has occurred. A further test of this hypothesis is to observe the net flux of water when the gut is bathed on both sides by identical solutions. Such experiments will demonstrate whether or not a net water flux can occur in the absence of osmotic and hydrostatic pressure gradients and they may also help to describe the relationship between salt and water transport.

Table 5 shows the average values of the observed net water fluxes from mucosa to serosa across small intestines bathed by identical media. In four pieces of tissue showing a net transport of water between identical Ringers the movement was

halted by the addition of 1 mM./l. potassium cyanide to the serosal medium. These results prove that there is a net uptake of water across the intestine when no bulk osmotic driving force exists across the tissue and that this transport is dependent, at least indirectly, on the metabolism of the tissue. Non-osmotic water flux evidently occurs in this tissue, however, under conditions where there is no net transport of salt.

Table 5. *Non-osmotic water flux between identical media*

Medium	Net water flux (mucosa to serosa) (μl. cm.$^{-2}$ hr.$^{-1}$) (mean ± S E.)
Ringer	8·1 ± 0·8 (83, 37)*
Sulphate Ringer	3·6 ± 0·5 (36, 9)
Choline Ringer	6·7 ± 0·6 (40, 8)

* The first number in each bracket denotes the number of measurements while the second denotes the number of pieces of intestine studied.

DISCUSSION

From the measurements of sodium and chloride fluxes across the small intestine of *Cottus* it is evident that the net transfer of these ions between identical Ringer solutions is achieved by some process whereby these ions are 'pumped' in the form of electrically neutral sodium chloride. This hypothesis is supported by two pieces of evidence: first, the absence of significant p.d.'s in Ringer, sulphate Ringer and choline Ringer, and, secondly, the absence of appreciable net transport of sodium and of chloride ions between sulphate and choline Ringers respectively. Diamond (1962*a*, *b*, *c*) concluded from experiments on the fish gall bladder that sodium and chloride ions were 'actively' transported across a cell membrane by a 'carrier' with specific sodium and chloride transport sites. He envisaged that this 'carrier' could cross the membrane only when both sites were occupied and this could produce a neutral transport of sodium chloride. Diamond also found that the gall bladder re-absorbed water, that the concentration of this absorbate was isotonic with Ringer and that the active transport of salt provided the driving force for water movement. On this point our experimental results diverge from Diamond's work since we have found that the absorption of water in *Cottus* intestine exists even in the absence of net salt transport. Conclusions drawn from our results are also at variance with those of Curran & Solomon (1957) on the *in vivo* rat ileum and also with those of Curran (1960) on an *in vitro* preparation of rat ileum. These workers considered that the active transport of sodium and chloride ions across the intestine produced an osmotic driving force for water movement; they found that the absorbates were approximately isotonic with Ringer, whereas in *Cottus* intestine the concentration of the absorbate passing between identical Ringers is about 1000 mM./l. (considerably hypertonic to Ringer). Thus although similar processes may appear, *prima facie*, to exist in the intestinal salt and water transfer in some vertebrates, a detailed analysis indicates that basic differences occur.

Our experimental results seem to point to the operation of a mechanism for water absorption in *Cottus* intestine completely different from that found in the other

vertebrates studied. Since we have discovered the presence of water transport in conditions where no net salt flux occurs, it may appear that this is strong evidence for the existence of an 'active water pump'. Recently, however, there have appeared several theoretical papers (Curran & McIntosh, 1962; Ogilvie, McIntosh & Curran, 1963; Patlak, Goldstein & Hoffman, 1963) which suggest that net flows of water may exist in epithelial membranes as a consequence of their asymmetrical structure. House (1964) has shown that by virtue of the asymmetrical nature of the permeability characteristics of frog skin a net flow of water may arise and that this flow has a similar magnitude and direction to that actually observed. Such a mechanism may also operate in the marine teleost gut and the interesting problem of testing this hypothesis remains to be investigated. An immediate direct consequence of such a model may be that the net salt flux across the intestine, bathed by identical Ringers, is created by a frictional drag on the salt by the moving water. Some evidence for the interaction of salt and water exists in the following calculation.

In the experiments where artificial sea water was placed in the lumen of the gut and Ringer bathed the serosal surface it was found that the net serosal to mucosal flux of water was about 20 μl. cm.$^{-2}$ hr.$^{-1}$. Assuming that this flow is osmotic and taking $L_p = 0{\cdot}22$ μl./cm.^{2} m-osmolar. hr. it can be calculated that the effective osmotic gradient across the gut is about 90 m-osmolar which is considerably lower than the apparent osmotic gradient (equal to about 410 m-osmolar when only the salt concentration gradient is considered). This argument may underestimate the effective osmotic gradient if water absorption (about 10 μl. cm.$^{-2}$ hr.$^{-1}$) still occurs when sea water is in the lumen; however, taking this into account we find that the effective osmotic gradient is about 140 m-osmolar—still significantly lower than the apparent gradient. Staverman (1951) has shown that the osmotic pressure exerted at a membrane by a solution containing a diffusible solute is less than the theoretical value; the reflexion coefficient, σ_s, for a given solute s and membrane is the ratio of the observed osmotic pressure to the theoretical van't Hoff value. Evidently the flow of water in 'leaky' membranes is determined by the following equation

$$J_v = L_p(\Delta_p - RT\sigma_s\Delta C_s), \tag{4}$$

where J_v is the net flux of water across the membrane, Δ_p the hydrostatic pressure (equal to zero here), ΔC_s the concentration gradient of the permeating solute, R the gas constant and T the absolute temperature. Using equation (4) to describe the osmotic flow of water into the luminal sea water (i.e. $L_p = 2{\cdot}4 \times 10^{-6}$ cm. sec.$^{-1}$ atm.$^{-1}$, $J_v = 8{\cdot}3 \times 10^{-6}$ cm.^{3} cm.$^{-2}$ sec.$^{-1}$, $RT = 2{\cdot}5 \times 10^4$ atm. cm.^{3} mole^{-1} and $\Delta C_s = 4{\cdot}1 \times 10^{-4}$ mole cm.$^{-3}$) we find a value of about 0·34 for the reflexion coefficient for salt in the intestine. Mathematical expressions for σ_s have been calculated for two special membrane models by Kedem & Katchalsky (1961) and by Dainty & Ginzburg (1963), and both formulae are formally identical to

$$\sigma_s = 1 - \frac{P_s\overline{V}_s}{RTL_p} - \alpha_s, \tag{5}$$

where α_s expresses a direct interaction between solute and water passing through water-filled pores in the membrane, $\overline{V}_s$ is the partial molar volume of the solute and P_s is the solute permeability coefficient when no volume flow takes place.

Equation (5) provides an easy test for the existence of any frictional interaction between salt and water as they pass across the intestine since Kedem & Katchalsky (1961) and Dainty & Ginzburg (1963) considered that if solute flow occurs in a capillary system in the membrane then

$$\sigma_s < 1 - \frac{P_s \bar{V}_s}{RTL_p}, \tag{6}$$

The only unknown quantity in the inequality (6) is P_s and it can be shown that if relation (6) does not hold then $P_{NaCl} > 1{\cdot}8 \times 10^{-3}$ cm. sec.$^{-1}$ which is an exceptionally large value for the salt permeability coefficient of an epithelial tissue; Diamond (1962*b*) calculated that $P_{NaCl} = 1{\cdot}9 \times 10^{-6}$ cm. sec.$^{-1}$ for fish gall bladder. From the data of sodium and chloride fluxes, serosa to mucosa, on *Cottus* it is possible to estimate P_{NaCl} from the Fick equation since no appreciable electric forces act on these ions. This calculation assumes that J_{sm} for sodium and chloride is purely passive and gives $P_{NaCl} \leqslant 10^{-5}$ cm. sec.$^{-1}$. The simplest conclusion is that relation (6) holds and that water and salt exert frictional drags on each other while traversing the intestine. More direct evidence is, however, required to substantiate this view.

We conclude that the characteristics of *Cottus* intestine, affecting movement both of water and of ions, enable it to perform an important role in the osmoregulation of the animal. There remain, however, several puzzling questions about the nature of intestinal transport of ions and water.

SUMMARY

1. The unidirectional sodium fluxes from mucosa to serosa and from serosa to mucosa have been studied in the isolated small intestine of *Cottus scorpius*, bathed in Ringer and sulphate Ringer, by use of the isotope ^{22}Na. In addition the unidirectional chloride fluxes have been studied in Ringer and choline Ringer using the isotope ^{36}Cl.

2. In Ringer the mean ± S.E. values of these fluxes have been found to be: for sodium, 8·4 ± 0·6 (serosa to mucosa) and 23·9 ± 0·7 (mucosa to serosa) μequiv. cm.$^{-2}$ hr.$^{-1}$ and for chloride, 10·5 ± 0·9 (serosa to mucosa) and 21·9 ± 0·9 (mucosa to serosa) μequiv. cm.$^{-2}$ hr.$^{-1}$. In sulphate Ringer the mean ± S.E. values for sodium were 4·5 ± 0·3 (serosa to mucosa) and 6·8 ± 0·6 (mucosa to serosa) μequiv. cm.$^{-2}$ hr.$^{-1}$ and in choline Ringer the mean ± S.E. values for chloride were 5·1 ± 1·0 (serosa to mucosa) and 5·4 ± 0·9 (mucosa to serosa) μequiv. cm.$^{-2}$ hr.$^{-1}$.

3. The respiration rates of pieces of intestine placed in Ringer, sulphate Ringer, choline Ringer and artificial sea water have been measured in a Warburg apparatus.

4. The mean ± S.E. values of oxygen consumption in these media have been found to be 0·4 ± 0·2 (Ringer), 0·3 ± 0·2 (sulphate Ringer), 0·3 ± 0·2 (choline Ringer) and 0·1 ± 0·2 (artificial sea water) μl. O_2/mg. dry weight.hr.

5. The electric potential differences between identical serosal and mucosal media bathing isolated intestines have been measured.

6. The mean ± S.E. values of the potential difference in Ringer, sulphate Ringer, choline Ringer and artificial sea water have been found to be +0·6 ± 0·3, +2·2 ± 0·2, +0·8 ± 0·3 and +1·3 ± 0·2 mV., respectively (mucosal medium taken as reference).

7. The hydraulic conductivity of the isolated intestine has been found by measuring the change in net water flux arising from the creation of an osmotic gradient across

this tissue. Net water flux was measured by the change in weight of normal filled sacs. The mean ± S.E. value for this parameter has been found to be 0·22 ± 0·05 μl./cm.^{2} m-osmolar. hr. (or 2·4 ± 0·6 cm. sec.$^{-1}$ atm.$^{-1}$).

8. It has been found that, when artificial sea water is placed in the lumen of the intestine bathed in Ringer, a net flow of water into the lumen occurs. This net water flux falls to zero at a time dependent on the luminal volume (to be diluted) and, thereafter, a net water flux (mucosa to serosa) is established.

9. The net water movement across isolated intestines bathed on both sides by identical media has been studied.

10. The mean ± S.E. values for net water flux (mucosa to serosa) have been found to be: in Ringer, 8·1 ± 0·8, in sulphate Ringer, 3·6 ± 0·5 and in choline Ringer, 6·7 ± 0·6 μl. cm.$^{-2}$ hr.$^{-1}$.

11. The results are interpreted as showing that the net transfer of sodium and chloride ions between identical Ringer solutions is achieved by some process whereby these ions are 'pumped' in the form of electrically neutral sodium chloride.

12. It is considered that there is some evidence for the existence of a frictional interaction between sodium chloride and water as they pass across the intestine.

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