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STUDIES ON SALT AND WATER BALANCE IN *MYXINE GLUTINOSA* (L.)

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The myxinoids are a marine group of cyclostomes whose blood shows some similarities to the sea water in which they live, even when they are moved to more dilute environments. Reviews by Morris (1960) and more recently by Robertson (1963) emphasize that the plasma is slightly hypertonic to the environment and that during the course of ionic regulation divalent ions are reduced in the plasma relative to sea water (Smith in Cole, 1940; Robertson, 1954; Bellamy & Chester Jones, 1961). Thus the main work of ionic regulation seems to fall on the tissues themselves. Here, potassium partly replaces sodium, whilst phosphate and perhaps amino acids take the place of chlorides (Robertson, 1960; Bellamy & Chester Jones, 1961). The osmotic deficit between the plasma and the tissues seems to be made up in part by unidentified organic acids and by large amounts of trimethylamine oxide which, according to Bellamy & Chester Jones, reaches concentrations comparable with those found in marine elasmobranchs.

The present study is mainly concerned with the mechanisms which regulate the ionic composition of the plasma. The main aim was to collect and analyse samples of plasma and urine from individual hagfish in order to see what part the kidney played in these processes. Secondary aims were to see if swallowing sea water and extrarenal excretion of ions formed a regular feature of the mechanism of water and ion balance as it does in marine teleosts (Smith, 1930) and in lampreys (Morris, 1958, 1960).

MATERIALS AND METHODS

Hagfish were obtained from the Gulmarfjord on the west coast of Sweden by means of baited traps. The animals were maintained at 5–7° C. in aquaria supplied with running sea water at the Marine Station at Kristineberg, where the experimental work was carried out. McFarland & Munz (1958) have shown that the Pacific hagfish (*Polistotrema stouti*) becomes abnormally hypertonic when handled because of the production of slime induced by handling the animals. The animals were left for 2 or 3 days to acclimatize to aquarium conditions before they were used for the present experiments.

Animals were anaesthetized in 4% urethane before blocking the gut at the anus and cannulating the urinary ducts. Hagfish are difficult to anaesthetize and substances like chlorbutol and M.S. 222 (tricaine) seem to have little effect; even urethane is slow acting (20–30 min.) and recovery times are equally slow. The gut was emptied and then blocked by a purse-string ligature inserted through the integument by means of a curved needle. The ligature was tied around the gut in front of the anus. Urine

was collected through cannulae drawn from stiff polythene tube. The cannulae emptied into a small removable polythene sac (capacity 1 ml.) blown from the same material. The separate openings from each mesonephric duct are very small and they are frequently difficult to find, so that the technique eventually developed for cannulation involved cutting through the ducts in transverse section by means of a shallow cut into the dorsal wall of the cloaca. The separate cannulae were then inserted into the ducts and sewn into position. Animals were left to recover from the visible effects of anaesthesia in running sea water and they were then transferred to 4 l. of static aerated sea water or to running sea water for the duration of the experiments which generally lasted about 24 hr. In the case of animals kept in static sea water, phenol red was added (25 mg./l.) to act as an indicator of water absorption from the intestinal tract (Smith, 1930; Morris, 1958).

Blood was collected from subcutaneous sinuses into chilled centrifuge tubes dusted internally with solid heparin. The cells were then separated from the plasma by centrifugation. The volume of urine and of gut fluid was estimated by weight and all samples were transferred to air-tight polythene containers which were maintained deep-frozen at a maximum temperature of $-30°$ C. until the samples were analysed. The majority of the analyses were performed at Nottingham.

Table 1. *Dilution scheme for urine*

(The table should be read from left to right starting at Ca^{2+})

	Initial amount (ml.) and dilution	Final amount (ml.) and dilution	Amount of aliquot (ml.) and approximate concentration (m-equiv./l.)
Ca^{2+}	0·2	2·0 of 1 :10	1·2 of 2·0
K^+	0·8 of 1 : 10	8·0 of 1 :100	3·8 of 0·1
SO_4^{2-}	2·0 of 1 : 100	2·0 of 1 : 100	1·0 of 0·6
Mg^{2+}	1·0 of 1 : 100	2·0 of 1 : 200	1·0 of 0·5
Cl^-	1·0 of 1 : 100	2·5 of 1 : 250	1·0 of 2·0
Na^+	0·2 of 1 : 100	6·0 of 1 : 3000	6·0 of 0·15

The problem of analysing small quantities of fluid (0·1–0·2 ml. in the case of most urine samples) for a range of ions was overcome by means of a method which involved accurate serial dilution of the samples to obtain the quantities and concentrations of fluids required by particular methods. Each batch of analyses from individual animals was standardized against an artificial sea water of known composition (Pantin, 1946) which was diluted at the same time and to the same extent as the samples. This not only minimized interference effects during flame photometry, but also provided convenient standards for other methods (SO_4^{2-}, Mg^{2+}, Cl^-). In the methods for chemical analysis given below only the dilution scheme used for urine is given in detail (Table 1). Since larger quantities of plasma, gut fluid and sea water were available, it was usually possible to perform these analyses in triplicate.

Sodium, potassium and calcium

These analyses were carried out by means of an EEL flame photometer. Plasma, sea water and gut fluid were diluted to 1:50 for potassium analyses.

Sulphate

Duplicate 1 ml. samples were titrated with 0·01 N-$BaCl_2$ delivered from an Agla syringe-burette using 1 ml. of ethanol saturated with tetrahydroxy-*p*-benzoquinone (B.D.H.) as an indicator. The titration was carried out in small test tubes in a 'Biochem' absorptiometer using a green filter. The solution was stirred continuously during the titration by means of a glass paddle attached to a vibro-stirrer (Morris, 1959) which was kept to one side of the light path through the test tube. The end-point was found at the point of inflexion which results when the volume of titrant is plotted against optical density.

Magnesium

This ion was estimated colorimetrically by the method of Orange & Rhein (1951), which utilizes the stable colour developed when magnesium complexes with Titan yellow in solutions containing polyvinyl alcohol. Serum samples were deproteinized by 20% trichloracetic acid and centrifuged to remove the precipitated protein before analysis. Standards for these samples were treated in the same way. Measurements of optical density were made in 1 ml. microcuvettes of 2 cm. path length in a 'Spekker' absorptiometer using a green filter (520 mμ).

Chloride

These analyses were performed in duplicate on 1 ml. samples by a potentiometric method which was essentially similar to that of Ramsay, Brown & Croghan (1955). Glacial acetic acid (0·2 ml.) was added to each sample which was titrated with 0·01 N-$AgNO_3$ delivered from an Agla syringe burette. The silver-silver chloride electrode formed the paddle of a vibro-stirrer which gave almost instantaneous mixing during titration and allowed the process to be carried out continuously (Morris, 1959).

Freezing-point depression

The method due to Johlin (1931) was used on undiluted samples of 0·3 ml. and above using a micro-Beckmann thermometer. The micro-freezing-point depression apparatus designed by Ramsay & Brown (1955) was used for smaller quantities of fluid.

Phenol red concentrations from sea water and gut fluid were compared in a Du Bosch colorimeter after developing the colour with a known volume of 0·1 N-NaOH.

Dry weights were determined on samples which were evaporated to dryness under infra-red lamps and heated for 6 hr. at 100° C.

Cytological material was preserved in Helly's fluid and then treated for 3 days in 2% potassium bichromate to preserve phospholipide. The Azan technique was used for general staining (Pantin, 1946), whilst the Kull method (Baker, 1946) was employed for demonstrating mitochondria. The periodic acid-Schiff method and Alcian blue (Pearse, 1960) were used as histochemical tests for mucopolysaccharide.

Water balance

Tonicity

Table 2 shows the relative freezing-point depression (Δ° C.) of the sea water and body fluids of three individual hagfish which were kept in static sea water during the experiments.

Table 2. *Freezing-point depression of the environment and body fluids of* Myxine glutinosa

Animal no.	Sea water (Δ° C.)	Plasma (Δ° C.)	Urine (Δ° C.)
12	1·90	2·00	1·87
17	1·84	1·88	1·84
26	1·89	1·81	1·82

The plasma is seen to be hyperosmotic to sea water in two of the three cases studied. These results agree with those of previous workers who show hagfish blood to be about 2% hyperosmotic to sea water (Dekhuyzen, 1904; Schmidt-Nielsen & Schmidt-Nielsen, 1923; Smith, 1932), and these also correspond with results obtained by Greene (1904) working on the Pacific species, *Polistotrema stouti*. McFarland & Munz (1958) claim that the blood serum in *Polistotrema* is isotonic within 0·7% in animals which are handled carefully to prevent slime production, which, according to them, makes the blood more markedly hypertonic. In the present series of experiments the animals were undoubtedly stimulated by the presence of the urinary cannulae and this may account for the apparent large difference (5%) to be seen in one case (animal 12). The occurrence of a plasma value which is hypotonic to sea water is less usual, though similar examples have been recorded in the literature by others (Smith, 1932; Schmidt-Nielsen & Schmidt-Nielsen, 1923).

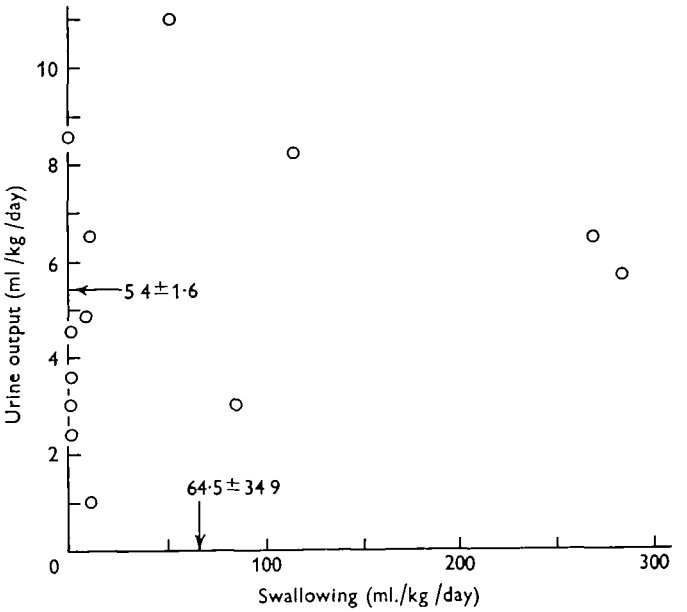
The urine appears to be hypotonic to the plasma in two cases and is close to isotonicity in the third. One of the difficulties in accumulating urine samples over long periods of time is that the urine must always partly reflect the immediate post-operative ionic composition of the blood at a time when the animal may be most heavily stressed. If *Myxine* behaves in the same way as *Polistotrema* and handling results in an increase of blood chloride, then this factor alone could account for a slightly hypertonic urine.

Urine output and swallowing

The data on urine output and swallowing are summarized in Fig. 1, and here thirteen successful urinary collections are recorded from a total of 45 attempts. The mean value of 5·4 ml./kg./day with a standard error of ± 1·6 indicates a low urine production which represents only 0·1 ml. or so for an average size animal of 60 g. The output is of the same order as that of marine elasmobranchs where the osmotic relationships are almost the same (Potts & Parry, 1964), and presumably indicates that the major part of the urinary output in *Myxine* may be obtained by osmosis from sea water into the blood.

The amount of sea water swallowed is very variable (Text-fig. 1) and the majority of animals did not swallow at all. The variation in swallowing shows that this is not a

regular and integral part of the water-balance mechanism as it is in lampreys and teleosts, whilst the lack of correlation between swallowing and urine output makes it unlikely that swallowing is generally used as a means of transferring water osmotically from the gut to the blood. This observation is supported by the fact that there was no apparent change in the concentration of phenol red in the gut of those animals which swallowed whilst kept in static sea water—an indication that the animals neither lost nor gained significant amounts of water in this way.



Text-fig. 1. Swallowing of water and output of urine in individual hagfish.

Ion balance

Plasma

The results of ionic analyses performed on the body fluids of six hagfish are summarized in Tables 3 and 5. The data have been calculated in mM./kg. water from dry-weight determinations which gave mean values of 988·7 and 921 g. water/l. for four serum samples and four sea-water samples respectively. The sea-water value has also been used to convert gut fluid and urine analyses into the same units. Since previous workers have taken hagfish from varying salinities, it is only possible to compare the ratios of plasma to sea water directly and these are given in bold figures in Tables 4 and 6.

The present series of analyses agree in general with those of others in that they show a reduction of divalent ions and an increase of sodium relative to sea water. A more detailed comparison shows discrepancies which suggest that in the present series animals were suffering from experimental stress brought about by anaesthesia, handling and cannulation. The main features of the effect of stress on ion balance seem to be:

(1) A tendency for the divalent ions, magnesium and sulphate to rise in the plasma. Animals kept in running sea water show higher average (plasma/sea water) % ratios for magnesium than the values recorded by other workers (Table 4, in bold figures),

Table 3. *The ionic composition of the external medium and body fluids of individual hagfish kept in running sea water*

	Quantity (ml./kg./day)	mM./kg. water								
		Na	K	Ca	Mg	Cl	SO_4	Cations	Anions	Total
Animal 29										
Sea water	—	455	9·4	9·8	52·6	524	27·1	526·8	551·1	1077·9
Gut fluid	865	455	9·0	8·5	48·8	481	28·1	521·3	509·1	1030·4
Plasma	—	540	12·7	6 6	23·9	544	11·3	583·2	555·3	1138·5
Urine	3·0	516	11·3	10·6	26·7	550	30·2	564·6	580·2	1144·8
Animal 36										
Sea water	—	455	9·4	9·8	52·6	524	27·4	526·8	551·4	1078·2
Gut fluid	Nil	—	—	—	—	—	—	—	—	—
Plasma	—	519	10·1	6·1	32·0	546	19·8	567·2	565·8	1133·0
Urine	1·1	448	15·8	8·9	37·6	524	21·3	510·3	545·3	1055·6
Animal 37										
Sea water	—	455	9·4	9·8	52·6	524	27·4	526 8	551·4	1078·2
Gut fluid	13·8	504	10·8	10·5	53·0	545	26·7	578·3	571·7	1150·0
Plasma	—	527	8·5	6 5	21·0	512	23·8	563·0	535·8	1098·8
Urine	6 4	478	11·2	7·2	24·8	535	15·3	521·2	550 3	1071·5

Table 4. *Concentration ratios for external medium and body fluids of individual hagfish kept in running sea water*

		Na	K	Ca	Mg	Cl	SO_4	Total
Animal 29	Gut fluid % / Sea water	100	96	87	93	92	104	96
	Plasma % / Sea water	119	135	67	45	104	42	105
	Urine % / Plasma	95	89	161	112	101	267	101
Animal 36	Gut fluid % / Sea water	—	—	Nil	—	—	—	—
	Plasma % / Sea water	114	107	62	61	104	72	105
	Urine % / Plasma	87	156	146	117	96	108	93
Animal 37	Gut fluid % / Sea water	111	115	107	101	104	97	107
	Plasma % / Sea water	116	90	66	40	97	87	102
	Urine % / Plasma	91	132	111	118	104	64	98
Smith* (in Cole, 1940)	Plasma % / Sea water	104	108	61	48	100	21	96
Robertson (1954)	Plasma % / Sea water	110	89	56	34	97	22	98
Bellamy & Chester-Jones (1961)	Plasma % / Sea water	117	91	61	38	102	—	106

* Recalculated into mM./kg. water using Robertson's (1954) figures of 921 and 989 g. water/l. for serum and sea water respectively.

whilst the sulphate ratios of the present series are twice to four times as great. The animals kept in static sea water have been affected to an even greater degree and in one case (animal 26), magnesium and sulphate values of the plasma actually exceed those of the external medium. The explanation of these results may be that the effect is

Table 5. *The ionic composition of the external medium and body fluids of individual hagfish kept in static sea water*

	Quantity (ml./kg./day)	mM./kg. water Na	K	Ca	Mg	Cl	SO_4	Cations	Anions	Total
Animal 12										
Sea water	—	464	9·4	9·6	52·5	535	27·8	535·5	562·8	1098·3
Gut fluid	114	468	9·3	10·5	46·5	521	26·6	534·3	547·6	1081·9
Plasma	—	480	15·1	3 2	50·1	570	19 7	548·4	589·7	1138·1
Urine	1·5	484	7·9	10·7	30 7	535	20·2	533·3	555·2	1088·5
Animal 17										
Sea water	—	464	9·6	9·9	52·5	540	26·7	536·0	566·7	1102·7
Gut fluid	278	348	8·2	7·2	53·5	400	22·3	416·9	422·3	839·2
Plasma	—	479	10·5	5·8	47·5	425	19·3	542·8	444·3	987·1
Urine	5·9	445	12·2	9·5	34·3	358	24·2	501·0	382·2	883·2
Animal 26										
Sea water	—	460	9·6	9 9	52·0	530	27·7	531·5	557·7	1089·2
Gut fluid	270	390	6·8	7·3	25·8	365	22·0	429·9	387·0	816·9
Plasma	—	455	10·6	5·7	54 4	505	20·0	525·7	525·0	1050·7
Urine	6·4	460	7·8	9·5	45·7	546	21·8	523·0	567·8	1090·8

Table 6. *Concentration ratios for external medium and body fluids of individual hagfish kept in static sea water*

		Na	K	Ca	Mg	Cl	SO_4	Total
Animal 12	Gut fluid % / Sea water	101	98	109	88	97	96	98
	Plasma % / Sea water	103	161	33	95	106	71	103
	Urine % / Plasma	101	52	334	61	94	103	97
Animal 17	Gut fluid % / Sea water	75	85	73	102	74	84	78
	Plasma % / Sea water	103	109	59	90	79	72	101
	Urine % / Plasma	93	122	164	72	84	125	92
Animal 26	Gut fluid % / Sea water	85	71	74	50	69	79	75
	Plasma % / Sea water	99	110	58	105	95	72	96
	Urine % / Plasma	101	74	167	84	108	109	99

due to the lingering action of urethane which would tend to accumulate in static sea water and hence prolong its effect. Indeed, it seems possible that urethane may act by interfering with magnesium balance (see below), allowing a high concentration of magnesium ions to accumulate in the blood stream.

(2) A second stress reaction, which has been mentioned already, is the way, in which hagfish respond to handling. Robertson (1963) has pointed out that both his own analyses (1954) and those of Bellamy & Chester Jones show *Myxine* plasma to be slightly hypotonic to sea water if the comparison is made in terms of the number of particles in solution. Using the same basis for comparison, animals from running sea water show degrees of plasma hypertonicity which vary from 2 to 6% (Table 3), and this is presumably a reflexion of the handling effect recorded on *Polistotrema* by McFarland & Munz. Of the animals maintained in static sea water, only one (Table 5, animal 12) shows similar reactions, presumably because the effect of the anaesthetic would be to reduce post-operative disturbances.

(3) A third feature which is worth noting is the high variability of plasma potassium in both groups of animals. Only one animal (Table 3, animal 37) shows the decreased plasma/sea water ratio shown by other workers.

Gut fluid

The relationships between the external medium, the gut fluid and the plasma (Tables 3 and 5) show the type of pattern which one might have expected in three out of the six cases studied. Animal 37 did not swallow, but in animals number 12, and 29 the total number of particles analysed in the gut fluid lies between that for sea water and plasma indicating that material has been transferred through the gut wall. The concentration of phenol red in the gut fluid of animal 12 was the same as that of sea water, so that in this case, at least, there was no significant movement of water. The results from both of these animals suggest a distribution of ions according to the diffusion gradient which must exist between the gut fluid and the plasma. Exceptions to this are gut sulphate in animal 29 (Table 3), and gut calcium in animal 12 (Table 5), both of which are higher in the gut than either the sea water or plasma. Potassium and chloride are lower in the gut than in sea water or plasma indicating the possibility of active transport mechanisms for both of these ions.

The relationships are much more difficult to interpret in the remaining three animals. Two of these (17 and 26) kept in static sea water have gut contents which are roughly equal to 75% sea water, whilst animal 37 shows a 10% increase in the ionic contents of its gut relative to sea water. These results are only explicable by assuming massive and unexpected movements of water or ions.

Urine

The total concentration of ions in the urine is lower than in the plasma in every case but animal 29 (Tables 3 and 5). These results, though incomplete in some respects (e.g. HCO_3^-, NH_3^+), tend to confirm the determinations of freezing-point depression (Table 2) and indicate that *Myxine* produces a slightly hypotonic urine.

Considering first the urinary composition of the more normal animals kept in running sea water, the most notable feature is the way in which the divalent ions calcium, magnesium and sulphate are excreted in the urine (Tables 3 and 4, (urine/plasma) % and this presumably accounts in part for their lowered ratios in the plasma. Animal 37 is unusual in that sulphate does not appear to be excreted. The fact that sodium is conserved and chloride excreted is also in accordance with the

normal plasma/sea-water ratios of these ions, remembering that some of the plasma chloride values in the present series are probably high because of handling.

The same sort of conclusions arise from considering the urinary composition of animals kept in static sea water (Tables 5 and 6), except that here sodium and chloride ratios tend to be more variable. There is an important difference, however, since magnesium no longer appears to be excreted by these animals. This is consistent with the high magnesium values in the plasma of these animals noted earlier and raises the question of whether the (urine/plasma) % values of less than 100 actually represent conservation of the ion in these cases. The concentrations of magnesium in the urine are in fact slightly higher than those from animals kept in running sea water (cf. Tables 3 and 5) so that it seems much more likely that the kidney is excreting magnesium at its maximum rate in response to a heavy magnesium load imposed from elsewhere. It is difficult to decide whether this arises from the tissues, where magnesium levels are about the same as the plasma (Robertson, 1954; Bellamy & Chester Jones, 1961) or from the external medium. If the effect is on the permeability of the external surfaces, it is certainly a very specific one, because the levels of calcium and sulphate excretion are relatively unaffected. It is interesting to note in this connexion that magnesium does seem to enter the gut faster than calcium and sulphate in two of the animals in question (Table 6, animals 12 and 26).

Potassium is the only ion which has not been considered so far, and in this case the (urine/plasma) % ratios indicate conservation in two cases and excretion in the other four. There seems little doubt that conservation is taking place in those cases where the (urine/plasma) % ratios are below 100 (animals 12 and 29) because the concentration of potassium in the urine is in fact lower than the surrounding sea water. The reasons for the variability in potassium excretion are perhaps not so clear, but they may be related to the previous nutritional state of the animal. The gut contents of those animals which were examined were very variable and consisted mainly of polychaete worms in individuals which had eaten recently. These might be expected to furnish quite concentrated sources of potassium.

Extra-renal ion exchange

The main sites of ion exchange between the animal and its external medium are the gut, the gills and the integument, apart, that is, from the kidney which has already been considered. The tissues can be neglected in this connexion because they are entirely surrounded by blood and they presumably maintain their characteristic ionic composition by active transport mechanisms and selective permeability. This means that under normal conditions the tissues will maintain a steady-state condition with the blood and should not therefore affect the ionic composition of the body fluids.

It is possible to calculate the quantity of ions which move from the sea water through the blood to the urine from the quantity and composition of urine passed during the day. In this way one can assess the relative permeabilities of the external surfaces to the various ions, taking into account the diffusion gradient which exists between the sea water and plasma. There are, however, two factors which are likely to give rise to errors in calculations of this sort, both of which can be traced to the results of experimental interference. The first of these concerns changes of blood composition during the experimental period which are bound to lead to large errors. These can only be

corrected if the original composition and the proportion of the body space occupied by particular ions are known. The second factor, which is even more difficult to correct for, is possible changes in the steady-state conditions which normally obtain between the blood and the tissues.

In spite of these difficulties it is worth while looking at a comparatively simple case like animal 36, where the situation is not complicated by a simultaneous movement of ions or water in the gut fluid. Table 7 summarizes the quantity of ions excreted in this case, together with some assessment of the direction and magnitude of the diffusion gradient between sea water and plasma. Calcium, magnesium and sulphate enter the animal along diffusion gradients of varying magnitude and the amount of ion excreted is of the same order when the values are corrected to allow for the diffusion gradient. The fact that the plasma/sea-water ratios for these ions are higher than those recorded by Robertson and by Bellamy & Chester Jones (Table 4) implies that the ions must have accumulated in the blood stream and thus the present assessment of ionic flux may be lower than one might normally expect.

Table 7. *Calculated values for the renal excretion of ions by a non-swallowing animal and their relationship to the ionic gradient*

(Values marked * indicate a higher concentration of ions in sea water than in the plasma. For further explanation see text.)

		Na	K	Ca	Mg	Cl	SO_4
Animal 36	Renal excretion (mM./kg./day)	0·493	0·107	0·009	0·041	0·576	0·023
	Ionic gradient (mM.)	64·0	0·7	3·7*	20·6*	22·0	7·6*
	Renal excretion / Ionic gradient (μM./mM./day)	7·7	24·8	2·6*	2·0*	26·2	3·0*

Turning now to the situation for monovalent ions it can be seen that considerable amounts of sodium, potassium and chloride enter against their diffusion gradients. Whilst it may be argued that sodium transport could take place against the diffusion gradient because of the Donnan situation operating across the external surface, this can hardly be the case for chloride and for potassium, and one must consider the possibility that the large amounts of monovalent ions which are necessary to bring the urinary composition to isotonicity are provided by active transport.

The gill epithelium of *Myxine* shows no sign of the mitochondria-rich type of cell which seems to be associated with ion transport in lamprey gills (Morris, 1957). Indeed, the only gill cells which contained mitochondria in quantity in hagfish gill are in an unsuitable position for contributing to ionic movement since they lie within the gill epithelium and rarely have a free border (Pl. I, fig. 3). A close study of these cells revealed that these were stages in mucous cell production. The cells show heavy periodic acid-Schiff staining in later stages of development and they eventually contribute to large islands of acid mucopolysaccharide contained within the gill epithelium (Pl. I, figs. 1, 2) which stain heavily with Alcian blue.

It is difficult to evaluate the role of the gut in these studies partly because of the difficulty of separating movements of ions and of water across the gut. This applies particularly to those animals kept in running sea water where it was impossible to use

the phenol red method to assess swallowing and water absorption. Phenol red measurements from the second group of animals indicated that little transfer of water takes place across the gut so that the reduced concentration of ions which occurs in some cases (animals 17 and 26) can only be accounted for by movement of ions from the gut to the blood. The amounts of ions involved are very large when compared with the renal output (Table 8) and this must account for the inability of these animals to maintain normal salt balance. It is therefore difficult to believe that *Myxine* has to cope with such large amounts of swallowed sea water and hence increase its problems of salt and water balance, and it seems much more likely that the excessive swallowing noted in some of these experiments was a result of the experimental interference rather than a feature of the normal mechanism.

Table 8. *Calculated values for renal excretion and the uptake of ions from the gut of animals which swallow sea water*

(Values marked † lost ions to the gut fluid. Values marked * indicate a higher concentration of ions in sea water than in the plasma.)

		Na	K	Ca	Mg	Cl	SO_4
Animal 12	Amount excreted (mM./kg./day)	0·73	0·012	0·016	0·046	0·800	0·030
	Gut uptake (mM./kg./day)	0·46†	0·011	0·102†	0·684	1·596†	0·136
	Ionic gradient (mM.)	16·00	5·7	6·4*	2 5*	35·00	8·1*
Animal 17	Amount excreted (mM./kg./day)	2·63	0·072	0·056	0·202	2·112	0·143
	Gut uptake (mM./kg./day)	32·25	0·389	0·750	0·278	38 920	1·223
	Ionic gradient (mM.)	15 00	0 9	4·10*	5·00*	15·00	7·4*
Animal 26	Amount excreted (mM./kg./day)	2·94	0·050	0·061	0·292	3·494	0·140
	Gut uptake (mM./kg./day)	18·90	0·756	0·702	7·074	44·550	1·539
	Ionic gradient (mM.)	5 00†	1·00	4·2*	2·4	25 00*	7·7*

DISCUSSION

The present studies confirm previous observations that *Myxine* maintains the ion and water balance of its body fluids by a relatively simple mechanism which seems to be primitive by vertebrate standards. The blood is slightly hypertonic to the external medium and this presumably accounts for the production of small amounts of near-isotonic urine. The main function of the kidney seems to be ionic regulation of the plasma and in particular it seems to be concerned with the excretion of divalent ions. The type of mechanism employed by *Myxine* is thus similar to, though more intensive than, that employed by marine crustacea like *Maia* (Robertson, 1939) and *Carcinus* (Webb, 1940) where the function of the excretory organ also appears to be to reduce the divalent ion concentration of the blood as a preliminary to the main task of providing the most suitable ionic composition for the tissues.

Robertson (1954, 1957) has argued from both physiological and palaeontological evidence that the vertebrates were originally a marine group and that the osmotic conditions in myxinoids are a primitive feature derived directly from chordate ancestors. These views contradict those of Marshall & Smith (1930) who favoured a freshwater origin for the early vertebrates and for the development of the vertebrate glomerulus. There has been increasing evidence to support Robertson's view (Denison,

1956; White, 1958; Chester Jones & Phillips, 1960; Morris, 1960) and, since *Myxine* has large segmentally arranged glomeruli (Goodrich, 1930; Gérard, 1943), it follows that the vertebrate glomerulus must also have been developed in the sea, not so much as a means of getting rid of excess osmotic water as Marshall and Smith proposed, but rather as an ion-regulating device. It was presumably later, when the vertebrates entered fresh water, that the glomerulus proved to be a useful adaptation to the altered osmotic circumstances.

SUMMARY

1. Measurements of freezing-point depression and chemical analysis have been made of the plasma and urine of *Myxine*.

2. The plasma is generally slightly hypertonic to sea water whilst the urine tends to be slightly hypotonic to the blood.

3. The urinary output is low ($5{\cdot}4 \pm 1{\cdot}6$ ml./kg./day) and the majority of animals do not swallow sea water.

4. Analyses of plasma and urine indicate that the kidney participates in ionic regulation by reducing the concentrations of calcium, magnesium and sulphate in the plasma relative to sea water. Chloride seems to be conserved whilst potassium may be conserved or excreted.

The high concentration of magnesium in the plasma of animals kept in static sea water may be caused by the after effects of urethane. These animals continue to excrete magnesium at normal rates.

5. The rates at which calcium, magnesium and sulphate enter an animal which does not swallow sea water are proportional to the diffusion gradients which exist between the external medium and the plasma. The situation is more complicated for monovalent ions, but there is no evidence of specialized ion-transporting cells within the gill epithelium.

6. In those animals which swallow sea water the amounts of ions absorbed from the gut are very large compared with the renal output and it would therefore seem unlikely that swallowing is part of the normal mechanism of salt and water balance.

7. It is argued that the mechanism of salt and water balance in *Myxine* is likely to be primitive and that the vertebrate glomerulus was probably developed originally in sea water as an ion-regulating device.

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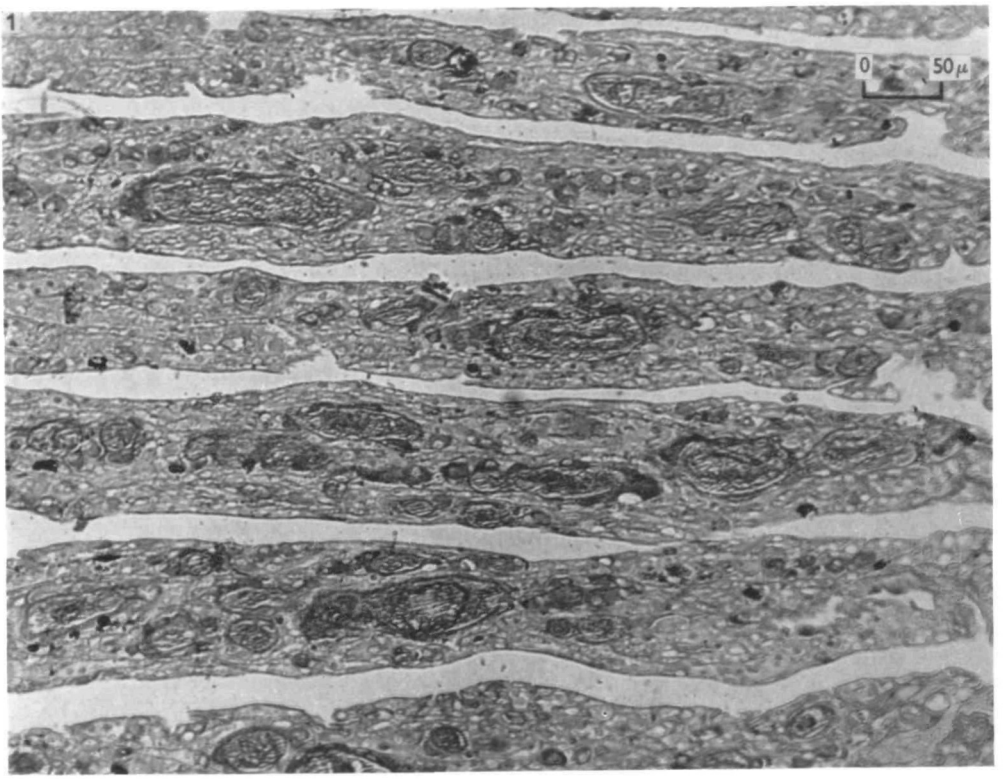
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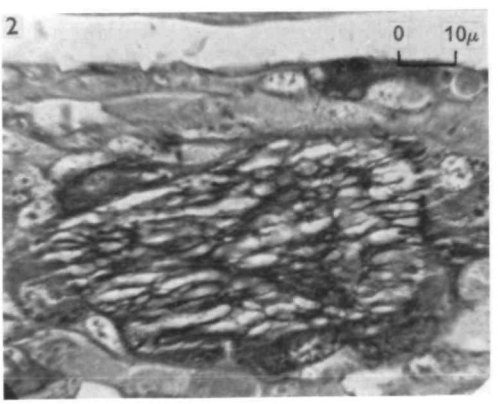
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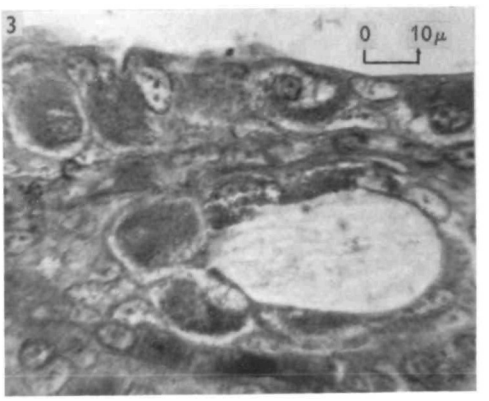
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EXPLANATION OF PLATE

Fig. 1. Low-power photomicrograph of a tangential section of a gill pouch of *Myxine* showing the distribution of mucous cells and islands of mucus Helly post-chromed; P.A.S. ×200.

Fig. 2. High-power photomicrograph of the same section showing mucous cells contributing to island formation Helly post-chromed; P.A S. ×700.

Fig 3. A similar area of gill showing the mucous cells stained heavily by the Kull method for mitochondria. Helly, post-chromed; Kull. ×700.