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OSMOREGULATION OF *LAMPETRA FLUVIATILIS* L. AND *PETROMYZON MARINUS* (CYCLOSTOMATA) IN HYPEROSMOTIC SOLUTIONS

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INTRODUCTION

Although osmotic and ionic regulation of Petromyzontia in fresh water has been carefully studied (Wikgren, 1953; Hardisty, 1956; Bull & Morris, 1967; Morris & Bull, 1968; Morris & Bull, 1970), there are still large gaps in our knowledge of the mechanism of 'marine' osmoregulation. This is because lampreys are only infrequently caught in the sea, at a time when they parasitize other fish. During their anadromous migration they can be caught in large numbers relatively easily, but their ability to survive in sea water is reduced (Fontaine, 1930; Galloway, 1933). However, Morris (1958) has shown that a small percentage of freshly caught, migrating river lampreys (*Lampetra fluviatilis* L.) can regulate their salt and water content successfully in 50% sea water, maintaining a lower blood osmotic pressure than the environment in a similar manner to marine teleosts. Water loss from the kidney is very small (1·3 ml/kg/day) and any osmotic loss of water at the body surface is corrected for by a swallowing mechanism with a consequent absorption of water by the intestine from the ingested fluid. Excess chloride in the blood is excreted extrarenally, almost certainly by large mitochondria-rich cells around the afferent vessels of the gills (Morris, 1957). These studies also indicated that a number of factors contributed to the breakdown of the 'marine' mechanism and these included an increase in permeability to water, loss of ability to swallow and replacement of 'chloride output cells' in the gills. The work was limited by the methods of analysis employed which consisted of measurements of net-water movement, and of freezing-point depression and chloride concentration of the body fluids. The present studies were designed to extend previous findings by employing more extensive analyses and in particular to discover the fate of divalent ions.

MATERIALS AND METHODS

Fourteen river lampreys (*L. fluviatilis*) and three sea lampreys (*Petromyzon marinus*) were used in this investigation. River lampreys were caught on their migration up the River Trent at Newark, Notts., and sea lampreys were kindly provided by the Ministry of Agriculture and Fisheries from their fish trap on the River Axe at Colyford, Devon. Between the time of capture and experiment (2–3 days), the animals were kept in large outdoor concrete tanks ($5' \times 5' \times 18''$) supplied with a constant flow of Nottingham tap water, and any visibly damaged animals were discarded.

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Experimental methods

The experiments were initiated by placing individual river lampreys in 6 l of aerated 33% sea water for 48 h in order to reduce the stress of abrupt transfer of an animal from fresh water to 50% sea water. At the end of this adaptation period the lamprey was anaesthetized in a 0·1 g/l solution of MS 222 (Sandoz) in 33% sea water (Thorson, 1959). After water had been shaken from the branchial pouches and residual gut fluid and urine had been removed by gentle abdominal massage, the animal was weighed in a tared conical flask. The urinary papilla was ligatured with Nylon thread and the alimentary canal was blocked at the posterior end by means of a Polythene plug. Animals were allowed to recover in fresh 33% sea water to remove the anaesthetic and were then transferred to 6 l of aerated 50% sea water containing 25 mg/l phenol red (Smith, 1930).

Sea lampreys were treated in a similar manner to river lampreys, but 76 l of 33% sea water and dyed 50% sea water were needed in view of the large size of the animals. They were weighed in a large net attached to the pan of an Ohaus Dial-o-gram balance.

After the experimental period of 24 h in 50% sea water, the animal was re-anaesthetized and weighed. Urine was collected from the urinary papilla by means of a fine glass pipette. The body cavity was opened up by a ventral longitudinal incision and the straight intestine was ligatured anteriorly and posteriorly prior to removal. The gut fluid was then carefully expelled from the isolated intestine into a capped Polythene tube. A blood sample was taken by heart puncture with a heparinized glass pipette. After centrifuging for 5 min at 4000 rev/min, as much as 0·4 ml of plasma could be obtained from a single lamprey. The weight change of the animal at the end of the experiment was corrected by subtracting the weight of urine plus gut fluid.

Net water movements in the animal were calculated by the method devised by Smith (1930) for teleost osmoregulation and used by Morris (1958) on the river lamprey. According to this method the concentration of phenol red in the gut fluid is proportional to the amount of water absorbed and thus the swallowing rate can be calculated, provided the animal does not absorb phenol red. There is no evidence of phenol red absorption by lampreys. Thus from a consideration of the volume of the gut fluid and the concentration of phenol red at the end of 24 hr in dyed 50% sea water, the weight change of the animal and the urine production, it is possible to analyse the net movement of water into: water swallowed by the lamprey, water absorbed by the intestine, water lost via the kidney and water lost at the body surface by osmosis.

Analytical techniques

All the fluids (plasma, urine, gut fluid and 50% sea water) were stored in sealed Polythene tubes at −30 °C until required for analysis. Sodium and potassium were determined by flame emission spectroscopy using the Unicam S.P. 90 Atomic Absorption Spectrophotometer. This instrument was also employed for the analysis of calcium and magnesium, but atomic absorption methods were used instead of flame emission.

Chloride was titrated against silver nitrate using the potentiometric method of Ramsay, Brown & Croghan (1955), and sulphate was titrated against barium chloride using tetrahydroxyparabenzoquinone as an indicator (Morris, 1965).

A colorimetric analysis, dependent upon the formation of molybdenum blue from a phosphomolybdic acid complex, was used for the determination of inorganic phosphorus (expressed as mM/l PO_4^{3-}). This particular analysis does not require previous de-proteinization of the sample and is supplied as a test kit by Schweizerhall, Chemical Works, Basle, Switzerland. However, it is not suitable for samples containing phenol red (gut fluid) because the dye seriously interferes with the colorimetric measurement of molybdenum blue.

Osmotic pressure was measured in terms of the freezing-point depression of the fluid (Δ °C) by the micro-method of Ramsay & Brown (1955).

By careful serial dilution of the sample it was possible to perform all these chemical analyses on as little as 0·05 ml of urine or plasma. The repeatability of the methods was assessed by ten analyses of a 50% sea water solution, and the standard error never exceeded 1·1% of the mean.

RESULTS

Freshwater animals

Analyses of the blood plasma of both species of lamprey maintained in fresh water are shown in Table 1 for comparison with animals from 50% sea water. Our results on individual *Lampetra fluviatilis* agree with those of Robertson (1954) obtained from a pooled sample, apart from inorganic phosphorous where we obtain a value which is closer to that reported by Fontaine (1932) for the marine lamprey.

Table 1. *The ionic composition of the blood plasma of lampreys in fresh water*

(Mean concn. of ion in mM/l ± S.E.)

Spp.	Source	Δ °C	Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-	PO_4^{3-}
Lampetra fluviatilis	Present series (9 animals)	0·505 ±0·008	119·6 ±3·95	3·9 ±0·29	2·5 ±0·36	2·0 ±0·33	103·6 ±4·07	2·0 ±0·37
	Robertson (1954) (8 animals)	—	114·7	3·1	1·9	2·0	92·0	6·7
Petromyzon marinus	Present series (4 animals)	0·495 ±0·010	111·9 ±1·80	2·3 ±0·32	1·8 ±0·010	1·5 ±0·05	99·6 ±2·57	2·5 ±0·29
	Fontaine, 1930, 1932	0·540	—	—	—	—	119·0	1·8

Table 2. *The change in ionic composition of the blood plasma of freshwater lampreys during the spawning migration*

Stage and number of animals	Δ °C	Na^+ mM/l	Cl^- mM/l
Fresh-run (6) (January)	0·451 ±0·008	120·5 ±1·6	95·0 ±1·9
Maturing (6) (March)	0·515 ±0·003	122·0 ±3·0	107·3 ±3·2
Mature (6) (May)	0·500 ±0·006	122·0 ±6·3	106·0 ±1·3
L.S.D. at $P = 0·01$	0·04	N.S.	7·8

Table 2 compares the levels of osmotically important ions at various stages of the spawning migration in *L. fluviatilis*, and shows that there is a significant increase in the freezing-point depression and chloride concentration as the animals mature in

fresh water. We think that this is brought about because the freshwater mechanism of osmoregulation is incompletely established in many fresh-run animals and this could explain the difference in ionic levels obtained by various workers. Thus, our complete analyses and those of Robertson may be characteristic of fresh-run animals, whilst those of Fontaine could well be characteristic of more mature animals.

'Marine' osmoregulation

Table 3 shows the results obtained from lampreys immersed in 50% sea water ranked in order of weight change recorded during the experiments. Various aspects of the osmoregulatory process are discussed separately below.

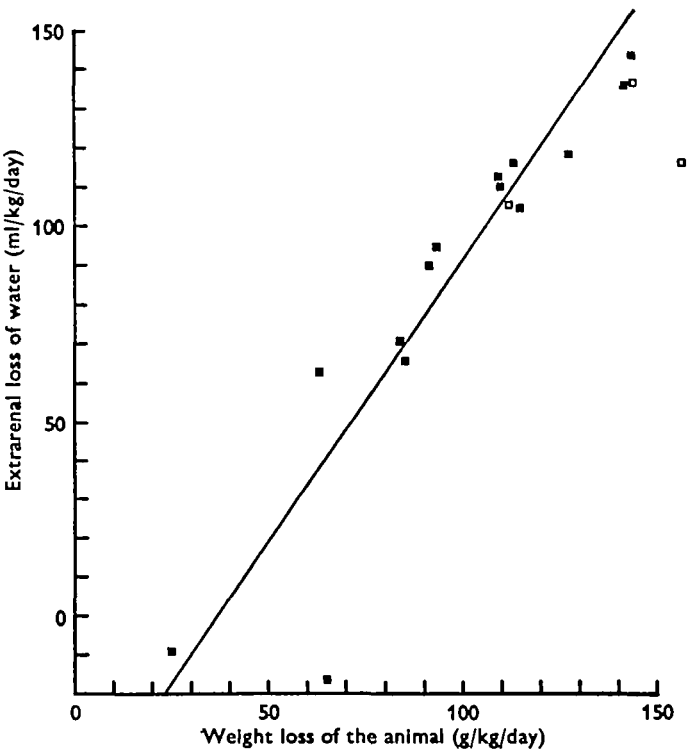

Fig. 1. The relationship between extrarenal water flux and weight loss of lampreys in 50% sea water. ■, *Lampetra fluviatilis*; □, *Petromyzon marinus*.

Water balance

Total weight change (compensated for gut content and urine) has been used as a criterion for water balance, and animals are considered to be osmoregulating successfully in 50% sea water when there is no weight loss. The high weight loss shown by many of the animals (Table 3, column 5) is the result of a breakdown of the mechanism of 'marine' osmoregulation as the animal migrates into fresh water (Morris, 1958). Perhaps the most important factor contributing to this weight loss is the extrarenal loss of water along an osmotic gradient. Fig. 1 shows the linear relationship between the osmotic loss of water from the animal and the total weight change, and reflects the

Table 3. *Water fluxes and ionic analyses of lampreys after 24 h in 50% sea water*

Col. 1 [redacted]	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
							Water absorbed													
Animal no.	Initial weight (g)	Final weight (g)	Time of expt. (h)	Weight change (g/kg/day)	Vol. of gut fluid (ml)	Phenol red conc.	Water swallowed	(ml/kg/day)	(%)	Urine vol. (ml)	Urine output (ml/kg/day)	Extra-renal loss (ml/kg/day)	Fluid	Δ°C (°C)	Na^+ (mM/l)	K^+ (mM/l)	Ca^{++} (mM/l)	Mg^{++} (mM/l)	Cl^- (mM/l)	SO_4^{--} (mM/l)	PO_4^{---} (mM/l)
Lampetra fluviatilis																				
1	62·9	63·5	24	+9·5	1·05	3·1	51·8	35·1	67·7	0	0	25·6	P.	0·555	155·4	6·2	2·9	4·4	131·8	—	1·9
													U.	—	—	—	—	—	—	—	—
													G.F.	0·530	64·1	8·6	17·7	92·6	143·4	63·1	—
2	82·6	83·6	18	+16·1	1·10	5·6	99·5	81·7	82·2	0	0	65·6	P.	0·630	171·7	8·0	4·3	9·0	140·9	—	5·7
													U.	—	—	—	—	—	—	—	—
													G.F.	0·600	48·9	7·3	28·1	176·9	177·2	90·9	—
3	55·1	51·6	24	−63·5	1·16	1·1	23·2	2·1	9·1	0·15	2·7	62·9	P.	0·875	221·7	9·9	3·0	6·1	215·3	—	2·3
													U.	0·805	172·2	5·9	6·4	104·1	217·4	51·0	0
													G.F.	0·810	200·0	3·3	5·2	31·6	227·5	13·5	—
4	69·6	65·0	24	−66·1	3·48	1·4	70·0	20·0	28·0	0·06	0·9	85·2	P.	0·840	200·0	6·6	3·0	6·4	166·8	—	—
													U.	0·680	189·7	5·7	6·0	79·0	219·1	34·7	0
													G.F.	0·640	153·3	2·5	7·9	42·2	195·2	18·2	—
5	27·9	25·9	24	−71·7	1·70	1·2	73·1	12·2	16·7	0	0	83·9	P.	0·725	—	—	—	—	—	—	—
													U.	—	—	—	—	—	—	—	—
													G.F.	0·810	181·5	3·8	5·8	24·7	206·9	14·6	—
6	51·1	46·5	24	−90·0	0·08	2·4	3·8	2·2	57·9	0·05	1·0	91·2	P.	0·850	—	6·5	2·9	—	—	—	—
													U.	0·785	138·2	4·4	4·7	43·6	186·5	—	0
													G.F.	0·475	—	—	—	—	—	—	—
7	51·9	46·6	26	−94·3	0·35	1·7	10·6	4·4	41·5	0·29	6·1	92·6	P.	0·680	182·6	5·7	2·8	3·7	168·6	—	2·8
													U.	0·635	104·3	2·7	5·0	104·1	194·0	45·2	0
													G.F.	0·635	152·2	6·0	10·4	51·0	189·0	—	—
8	58·8	52·2	25·5	−105·6	0·14	5·5	12·3	10·0	81·3	0·10	1·6	114·0	P.	0·765	187·0	6·5	2·8	4·4	165·3	—	3·3
													U.	—	64·9	2·9	6·6	78·2	230·3	26·3	0
													G.F.	0·430	—	—	—	—	—	—	—
9	79·6	69·9	26·5	−110·4	0·27	1·7	5·2	2·2	42·3	0·25	2·8	109·8	P.	0·685	176·1	6·1	3·3	4·4	173·7	—	5·5
													U.	0·525	80·9	3·9	6·0	73·3	182·4	32·8	0
													G.F.	0·610	—	—	—	—	—	—	—
10	58·4	51·3	26	−112·2	0·26	1·7	7·0	2·9	41·4	0·39	6·2	108·9	P.	0·745	187·0	6·0	3·3	4·7	164·4	—	3·8
													U.	0·515	57·4	2·7	0·9	30·0	203·3	24·0	0
													G.F.	0·625	—	—	—	—	—	—	—
11	59·5	52·6	24	−116·0	0·15	1·1	2·8	0·3	10·7	0·24	4·0	112·3	P.	0·780	179·3	6·9	2·7	5·1	166·9	—	—
													U.	0·695	160·9	3·1	6·6	37·0	172·8	42·1	0
													G.F.	—	—	—	—	—	—	—	—
12	63·3	55·8	24	−118·5	1·70	1·4	37·6	10·7	28·5	0·14	2·2	127·0	P.	0·810	169·6	4·4	2·4	3·4	166·7	—	2·4
													U.	0·785	145·7	3·7	5·2	17·5	168·5	16·8	—
													G.F.	0·715	154·9	2·8	6·9	36·0	191·8	19·2	—
13	68·3	59·0	24	−136·1	0·50	2·0	14·6	7·3	50·0	0·10	1·5	141·9	P.	0·725	179·3	5·0	2·4	4·2	166·5	—	2·6
													U.	0·710	47·8	1·5	22·1	44·2	131·3	13·5	—
													G.F.	0·735	140·2	2·8	8·3	56·6	192·7	23·3	—
14	72·9	61·1	27	−143·9	0	—	0	0	—	0·10	1·2	142·7	P.	0·780	187·0	5·5	1·8	—	151·1	—	—
													U.	0·530	109·6	4·7	3·5	20·4	140·3	6·9	0
													G.F.	—	—	—	—	—	—	—	—
Petromyzon marinus																					
15	807·0	713·0	24	−116·5	8·64	5·0	53·5	42·8	80·0	1·23	1·5	157·8	P.	0·750	191·3	4·0	2·6	3·4	172·6	—	3·1
													U.	—	—	—	—	—	—	—	0
													G.F.	0·675	28·8	5·8	18·8	123·4	147·8	76·5	—
16	839·0	748·0	24	−106·0	1·71	4·7	9·6	7·5	78·2	0·07	0·1	113·4	P.	0·745	177·2	3·3	2·3	3·2	167·0	—	2·2
													U.	0·600	—	—	—	—	—	—	—
													G.F.	0·720	40·2	5·4	14·1	123·4	146·4	86·3	—
17	591·5	510·5	24	−137·0	2·19	2·5	9·2	5·4	58·7	0·30	0·5	141·9	P.	0·805	193·5	4·4	2·5	4·0	172·6	—	5·1
													U.	0·615	89·1	4·4	12·5	43·2	118·0	75·7	0·7
													G.F.	0·630	85·9	5·5	12·5	66·6	164·6	37·2	—
													50% sea water	1·010	226·5	5·0	5·0	26·5	262·2	14·1	0

P. = Plasma; U. = Urine; G.F. = Gut fluid

increase in the permeability of the animal to water, which occurs during its anadromous migration (Morris, 1956, 1958).

As compensation for this extrarenal loss of water, both species of lamprey swallow large quantities of 50% sea water (up to 99·5 ml/kg/day) from which as much as 82·2% of the ingested water is absorbed by the intestine (Table 3, column 10). The ability to swallow water is reduced during the migration and also contributes towards the failure of the majority of lampreys to maintain a constant weight in 50% sea water. Fig. 2 illustrates the relationship between the volume of water swallowed and the weight loss of the animal. Although the correlation is not as well defined as that between extrarenal water flux and weight loss, it is sufficiently good to show that the swallowing mechanism is reduced in animals that cannot maintain water balance.

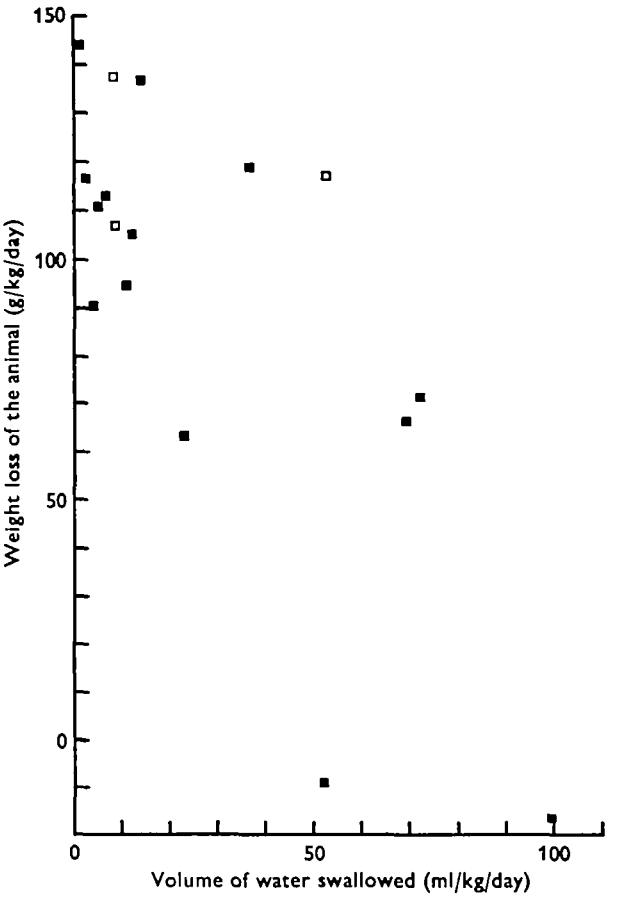

Fig. 2. The relationship between the volume of sea water swallowed and weight loss of lampreys in 50% sea water. ■, *Lampetra fluviatilis*; □, *Petromyzon marinus*.

According to Berg (1931) and Pietschmann (1933), the alimentary canal of *L. fluviatilis* degenerates during the anadromous migration and closes over in the anterior region. It has been suggested (Koch, 1968) that this degeneration may be responsible for the breakdown and loss of the swallowing mechanism. In order to test this hypo-

thesis, pieces of alimentary canal from the region of the pancreas were fixed in Bouin's fluid, embedded, transversely serially sectioned and stained with Ehrlich's acid haematoxylin and eosin. The cross-sectional surface area of the gut lumen was measured at intervals of 500 μm by tracing the image of the gut lumen from a projection microscope, and weighing the tracing. In all the animals studied, the narrowest point of the alimentary canal was found to be at the region of the cranial cord of the follicles of Langerhans (see Barrington, 1944). However, none of the animals showed signs of closure in this region and no correlation could be found between the narrowest point of the gut and the volume of 50% sea water swallowed (Table 4). It appears, therefore, that the swallowing mechanism is lost before degeneration and occlusion of the alimentary canal.

Table 4. *The volume of* 50% *sea water swallowed and the cross-sectional area of the narrowest point of the alimentary canal in* Lampetra fluviatilis

Animal no.	Weight (g)	Water swallowed (ml/kg/day)	Cross-sectional area arbitrary units	Area/weight arbitrary units/100 g
4	69·6	70·0	105	151
1	62·9	51·8	239	379
8	58·8	12·3	450	765
7	51·9	10·6	328	632
9	79·6	5·2	183	230
14	72·9	0	481	660

The absolute amount of water absorbed from the gut fluid is of necessity dependent upon the volume of water swallowed by the animal, but when the absorbed water is expressed as a percentage of the swallowed water (Table 3, column 10) and compared with the volume of water swallowed (Table 3, column 8), it is clear that the absorption mechanism is independent of the swallowing mechanism. For example, lampreys 1 and 5 swallowed similar amounts of sea water, but the percentage absorption of the ingested fluid for animal 1 was over four times as high as for animal 5.

The urine production ranged from 0 to 6·2 ml/kg/day, which is extremely low when compared with the urine output of lampreys in fresh water (*Petromyzon marinus*, 159 ml/kg/day, Sawyer, 1955; *Lampetra fluviatilis*, 362 ml/kg/day, Wikgren, 1953; 160 ml/kg/day, Morris, 1956) and argues for an efficient renal conservation of water. The volume of the urinary tract of the river lamprey is 1–2 ml (Morris, 1958) and as the maximum volume of urine produced was 0·39 ml it is unlikely that back pressure on the kidney, as a result of the ligature on the urinary papilla, had any effect on reducing urine output.

Ion balance

The osmotic pressure and salt content of the plasma of lampreys from 50% sea water (Table 3) is significantly higher than that of animals maintained in fresh water (Tables 1, 2). This indicates that the majority of animals are unable to regulate the salt content of their body fluids successfully in 50% sea water. As ion balance is intimately associated with water balance, and as it has already been shown that the majority of animals lose weight in 50% sea water, this inability to maintain a low plasma osmotic pressure is to be expected. However, in the two animals which can maintain water balance (Table 3, animals 1 and 2), the osmotic pressure and salt

concentration of the plasma are still slightly elevated. Morris (1958) reports similar osmotic pressure values for animals osmoregulating successfully in 50% sea water (Δ = 0·572 °C) when compared with freshwater plasma levels (Δ = 0·456 °C). Burian (1910) reported a figure of 0·59 °C. for the blood of a sea lamprey caught in the Mediterranean, which can be compared with the mean figure of 0·495 °C for the blood plasma of sea lampreys caught in the River Axe. It appears, therefore, that lampreys tolerate a slight increase in blood osmotic pressure even when osmoregulating successfully in hyperosmotic media.

Absorption of ions by the intestine

From Table 2 it can be seen that the ingested 50% sea water at the end of the experiment (gut fluid) has a very different ionic composition from the environment. In lampreys with a well-developed water-absorption mechanism (animals 1, 2, 13, 15, 16, 17), the sodium concentration in the gut fluid is always lower than that in the blood, showing that sodium can be transported across the intestinal epithelium against a concentration gradient. There is also evidence that potassium can be moved against a concentration gradient because the concentrations in the gut fluid of animals 3, 4, 12 and 13 are lower than those in the plasma at the end of the experiment. However, movement of ions against a concentration gradient may not indicate active transport since the ions could be moving with an electrochemical gradient. Such a gradient might result from a Donnan situation between the blood and the gut in the present circumstance, leaving the blood negatively charged because of indiffusible anions and movement of chloride from gut to blood. This seems an unlikely explanation because the chloride levels in the gut of *Petromyzon marinus* are lower than in the blood and previous studies on *Lampetra fluviatilis* also show that chloride can be transported against a concentration gradient. Thus there is evidence that sodium, potassium and chloride can be transported against a diffusion gradient during the later stages of absorption, so that the gut fluid is usually hypo-osmotic to the blood at this time (Table 3, column 15). Under these circumstances water absorption can be accounted for by osmotic flux from the gut to the blood. There is also evidence (animals 5 and 13) that water absorption can also take place when the gut fluid is hyperosmotic to the blood and this may be the more normal circumstance in osmoregulating animals swallowing sea water.

The marked increase in the concentration of divalent ions in the gut (Table 3) indicates that the intestine is less permeable to divalent ions than to monovalent ions. Since the volume and ionic composition of both the 50% sea water swallowed and the gut fluid at the end of the experiment is known, it is possible to calculate the amount of any particular ion transported across the intestine. Fig. 3 shows the relationship between the movement of monovalent ions (Na^+ and Cl^-), divalent ions (Mg^{2+} and SO_4^{2-}) and water by the intestine. Ion movements are expressed as a percentage of the ingested ions. A regression line ($y = -1{\cdot}89x + 155{\cdot}6$) with a correlation coefficient of $-0{\cdot}55$ has been plotted for the combined Mg^{2+} and SO_4^{2-} data. The negative values for the percentage absorption of these ions by the intestine of certain lampreys indicates that the total amount of Mg^{2+} or SO_4^{2-} in the gut fluid was greater than that swallowed as 50% sea water. This phenomenon, together with the negative

gradient of the regression line, suggests that divalent ions have been secreted into the gut fluid by the intestine, particularly in animals that possess a well-developed water-absorption mechanism.

Urinary excretion of ions

The small volume of urine produced by lampreys in 50% sea water (up to 6·2 ml/kg/day) is always hypo-osmotic to the blood (Fig. 4), although the osmotic pressure (Δ = 0·515–0·805 °C) is very much higher than that of the urine of river lampreys in fresh water (Δ = 0·077 °C, R. Morris, unpublished). Despite the wide variation in

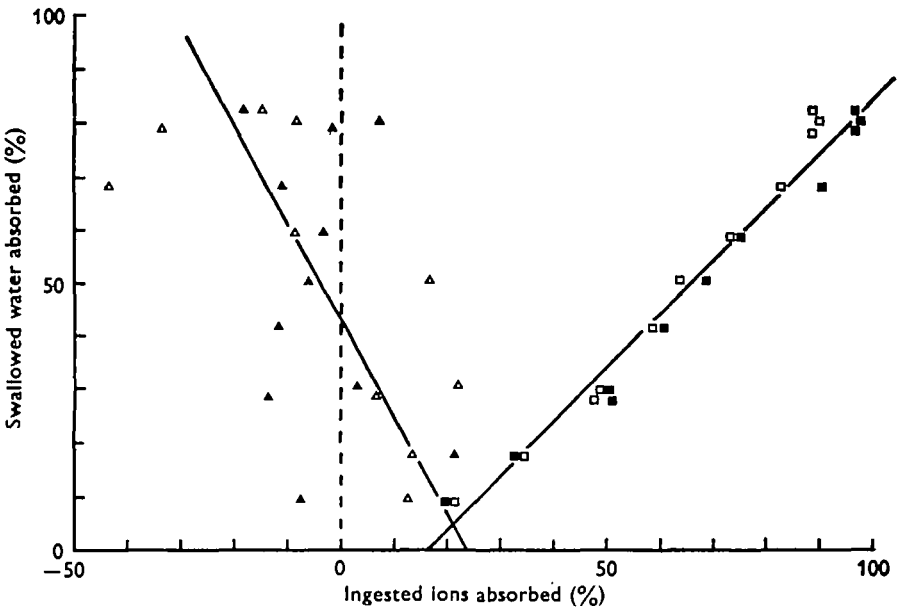

Fig. 3. The relationship between absorption of water and of monovalent and divalent ions by the intestine of *Lampetra fluviatilis* and *Petromyzon marinus*. □, Chloride; ■, Sodium; △, Sulphate; ○, Magnesium.

osmoregulatory ability of lampreys in 50% sea water, the mean ionic composition of the blood and urine are significantly different (Table 5).

The urine/plasma ratios based on these figures indicate that sodium and potassium are conserved by the kidney, whilst chloride is excreted at roughly the same concentration as that in the blood. The divalent ions calcium, magnesium and sulphate are all excreted, magnesium and sulphate reaching very high concentrations in the urine. Thus lampreys appear to be similar to marine teleosts in that the limited urine flow is used for the excretion of divalent ions from the blood.

Extrarenal excretion

Morris (1957) suggested that large mitochondria-rich cells found around the afferent vessels of the gills of a small percentage of freshly caught river lampreys are responsible for the active excretion of excess monovalent ions from the blood stream. In the present investigation the only lampreys to possess these cells (Table 3, animals

1 and 2) were also the only animals considered to be ion-balancing successfully. Since both animals absorbed large quantities of monovalent ions from the gut fluid yet neither produced any urine during the experimental period, it now seems almost certain that the so-called 'chloride cells' of the gill are in fact concerned with the extrarenal excretion of monovalent ions.

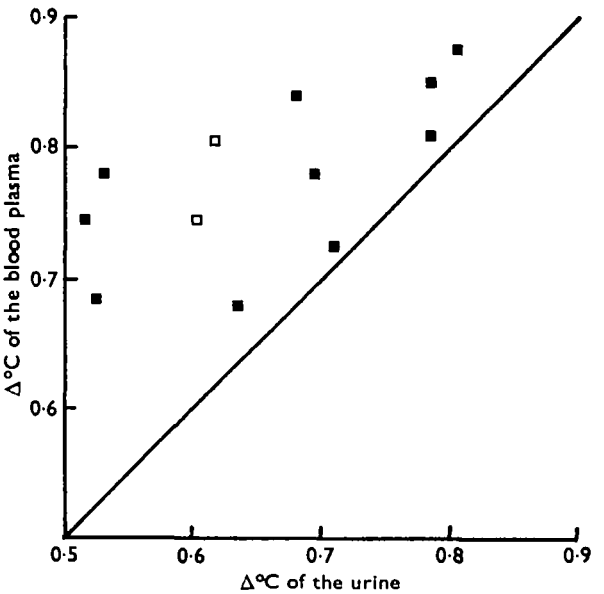

Fig. 4. The freezing-point depression (osmoticity) of the blood and urine of lampreys in 50% sea water. ■, *Lampetra fluviatilis*; □, *Petromyzon marinus*.

Table 5. *The ionic composition of the blood plasma and urine of lampreys after 24 h in 50% sea water*

(Combined results from both species; mean concn. of ion in mM/l ± S.E.)

Fluid	Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-	SO_4^{2-}
Blood Plasma (12 animals)	183·9 ± 3·07	6·0 ± 0·39	2·8 ± 0·12	4·8 ± 0·40	166·0 ± 4·52	2·6*
Urine (12 animals)	113·4 ± 13·11	3·8 ± 0·32	7·1 ± 1·44	56·2 ± 8·41	180·3 ± 10·59	33·5 ± 5·63
U/P ratio	0·617	0·633	2·536	11·700	1·086	12·880

* Taken from Robertson, 1954. *L. fluviatilis* in fresh water.

These 'chloride output cells' disappear from the gills at an early stage in the migration and this seems to be a major factor in the breakdown of 'marine' osmoregulation. Smaller, mitochondria-rich 'chloride uptake cells' (Morris, 1957) were not found in any of the fresh-run animals used in this investigation but were present in maturing river lampreys kept in fresh water for 2–3 months after capture. This may be taken as further evidence for the function of these cells, since, as pointed out earlier (Table 2), the blood osmotic pressure of fresh-run river lampreys is significantly lower than that of maturing animals.

DISCUSSION

There has been no evidence to suggest any fundamental differences in physiology between the two species of migratory lamprey used in this investigation, although only three *Petromyzon marinus* were used. The most striking feature is the close similarity between the methods of 'marine' osmoregulation of lampreys and teleosts.

In 50% sea water lampreys maintain their blood osmotic pressure below that of the environment by a combination of ion regulation and water regulation. They achieve water balance by swallowing sea water and then absorb water from the intestine. Water absorption, often against an osmotic gradient, is dependent upon the active absorption of monovalent ions (sodium, potassium and chloride) and compensates for the extrarenal osmotic loss from the animal. The gut residue is rich in the poorly absorbed divalent ions calcium, magnesium and sulphate. Furthermore, in many fresh-run lampreys, the total quantities of magnesium and sulphate in the gut fluid at the end of the experiment are higher than those ingested as 50% sea water. This suggests that secretion of divalent ions into the gut lumen against a concentration gradient may be part of the normal mechanism of 'marine' osmoregulation in lampreys. A similar result has been reported by Dall & Milward (1969), working with the marine teleost *Pelates quadrilineatus* (Bloch). They concluded that the swallowing mechanism may also serve for the elimination of calcium which is excreted into the gut lumen. However, further experimentation on this mechanism is needed in lampreys before the excretory role of the alimentary canal can be confirmed.

The urine is hypo-osmotic to the blood and the low flow rate (0–6·2 ml/kg/day) cannot account for the elimination of absorbed monovalent ions, indicating that these are excreted extrarenally by the gills. Bentley & Follett (1963) showed that the urinary output decreased from the normal freshwater level when river lampreys were immersed in hypotonic saline. Urine production decreased because of a reduction in glomerular filtration rate and the proportion of tubular re-absorption of water thus remained relatively constant. This may account for the low values of urine output in our experiments, where the relationship is also presumed to be between water availability and glomerular filtration rate. The kidney also excretes divalent ions in large amounts (Table 3). Using the values for glomerular filtration rate obtained by Bentley & Follett (1·6 × urine output) and allowing for the tubular re-absorption of water which occurs, we calculate that divalent ions must have been secreted by the kidney tubules. This is particularly interesting because of the presence of blind, aglomerular diverticuli in the nephron unit of the lamprey kidney (Regaud & Policard, 1902; Morris, 1960) which may represent a specific adaptation for this purpose.

Analysis of 'marine' osmoregulation in teleosts was initiated by Smith (1930) and has since been the subject of intense research (see reviews by Maetz, 1968; Potts, 1968). From the present investigation, the mechanism employed by lampreys to achieve salt and water balance in hyperosmotic solutions is similar in almost every respect to that of marine teleosts, and contrasts sharply with the isosmotic osmoregulatory mechanism of the other recent cyclostome group, the Myxinoidea (Morris, 1965). In view of this similarity, it is of interest to review briefly the evolutionary relationships of lampreys and teleosts.

From a critical analysis of vertebrate remains from Ordovician and Silurian deposits,

it has been suggested that the Agnatha–Gnathostomata transition occurred in the Ordovician period (about 500 million years ago) or even earlier (Colbert, 1955; Carter, 1967). Since the agnathous condition of lampreys is considered to be a primitive character, it follows that the evolutionary lines leading to modern lampreys and teleosts have been independent for at least 500 million years. It is now widely accepted that the earliest Ordovician vertebrate remains were marine, and a marine origin of the vertebrates has been proposed (Robertson, 1957). It is considered that the high osmotic pressure and salt content of the blood of the Myxinoidea is representative of the primitive marine osmoregulatory mechanism employed by the early vertebrates (Robertson, 1957; Morris, 1960).

The marine osmoregulatory mechanism of lampreys and teleosts is undoubtedly a reflexion of a freshwater stage during the evolutionary histories of the two groups. Since the division of vertebrates into Agnatha and Gnathostomata probably occurred in the Ordovician period or earlier, a time at which Robertson believes vertebrates to have been a primitive marine group of animals probably osmoregulating in a manner similar to the present-day myxinoids, it follows that the mechanism of 'marine' osmoregulation in lampreys and teleosts must have arisen independently. Parallel evolution in two groups that have been separate for at least 500 million years has resulted in almost identical physiological mechanisms.

SUMMARY

1. Freshly caught migrating lampreys were placed in 50% sea water and their method of osmoregulation was analysed. Some osmoregulated more successfully than others.

2. Water balance is maintained by a mechanism involving the drinking of large quantities of water (up to 99·5 ml/kg/day). Sodium, potassium and chloride are absorbed by the intestine (often against a concentration gradient) with the subsequent uptake of water. Divalent ions are not readily absorbed by the intestine and there is some evidence for the secretion of magnesium and sulphate into the gut lumen.

3. The limited urine flow (up to 6·2 ml/kg/day) is used for the excretion of calcium, magnesium and sulphate in high concentrations, but the urine is never hyperosmotic to the blood. The urinary excretion of monovalent ions is not sufficient to eliminate those entering by the intestine and extrarenal excretion at the gills must presumably occur.

4. The breakdown of this osmoregulatory mechanism during the anadromous migration involves: an increase in the permeability of the integument to water, a breakdown of the swallowing mechanism which is not dependent upon the occlusion of the alimentary canal, a reduction in the ability to absorb monovalent ions and water from the ingested 50% sea water, and a loss in the large mitochondria-rich 'chloride output cells' of the gills.

5. The similarities between the mechanisms of 'marine' osmoregulation of lampreys and teleosts are discussed in terms of the evolution of the two groups, and it is concluded that almost identical osmoregulatory mechanisms have evolved independently.

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