THE MECHANISM OF MARINE OSMOREGULATION IN THE LAMPERN (*LAMPETRA FLUVIATILIS* L.) AND THE CAUSES OF ITS BREAKDOWN DURING THE SPAWNING MIGRATION

By R. MORRIS

From the Department of Zoology, The University, Nottingham

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INTRODUCTION

An earlier contribution (Morris, 1956) described the osmoregulatory responses of lamperns to sea water during the time that the animals are engaged on a spawning migration into fresh water. Only fresh-run animals were found to be able to osmoregulate in dilute sea-water solutions hypertonic to their blood, and even in these creatures the osmotic responses were very variable. These facts suggested that the mechanism responsible for maintaining the blood hypotonic to sea water was breaking down as the need arose for the development of a new osmoregulatory mechanism to allow the animal to exist in fresh water.

The present study describes the osmoregulatory processes of fresh-run animals which were found to be capable of maintaining ion and water balance in dilute sea water and also attempts to analyse the reasons for the breakdown of the mechanism which takes place in other fresh-run animals. There were indications from previous work that the mechanism is similar to that already described for marine teleosts by Homer Smith (1930). Because of this, the experiments to be described were mainly designed to test whether the lampern was able to swallow sea water and excrete chloride extra-renally.

The gill epithelia of some fresh-run lamperns contain cells which are similar to those described from the gills of marine teleosts as chloride excretory cells, and in the lampern these cells are replaced by others as the animal matures (Morris, 1957). An attempt has been made to assess the number of chloride excretory cells which were present in individual experimental animals and this assessment has then been related to the osmotic responses of the animal.

MATERIAL AND METHODS

The experiments were conducted on a single haul of lamperns taken from the River Trent as early as possible in their spawning migration to ensure that a large proportion of animals capable of osmoregulating in sea water would be present. The animals were transported in damp grass from Newark-upon-Trent to Nottingham as soon as possible and those showing the slightest sign of damage were discarded.

The experimental procedure outlined below was successfully completed on

eighteen animals. These were taken in groups of five or six individuals, since this was the most convenient number to handle at one time. All experiments were completed within a month from the time the animals were captured.

Animals were first adapted to 33% sea water for a minimum period of 40 hr. They were weighed before and after this period to obtain a preliminary assessment of their osmoregulating capabilities from the extent to which they were able to maintain water balance in a solution which is only slightly hypertonic to their blood. They were then anaesthetized in chlorbutol prior to their swallowing-capacities being tested by the method devised by Smith (1930). According to this method the animal is immersed in a solution of a dye which cannot be absorbed by the gut, the gills or the skin. The gut is blocked to prevent loss of swallowed water, and after a suitable period of time the amount of water swallowed and absorbed can be estimated by comparing the concentration of dye in the gut residue with that in the surrounding sea water. The contents of the gut and kidneys were expelled from the lamperns by gentle abdominal massage, and the hind end of the gut was then blocked by inserting a solid cone of soft Polythene through the anus into the gut. At the same time the urinary papilla was ligatured for urine collection during the period of the experiment. This method was chosen both for its simplicity and because the urine output is known to be low when animals are immersed in hypertonic solution (Morris, 1956). The method is only valid for low urine outputs because the volume of the urinary tract of the lampern is small (1–2 ml.).

The animals were allowed to recover in 33% sea water before they were transferred to a solution of 50% sea water containing phenol red (25 mg./l.). After about 24 hr. they were again anaesthetized and weighed, and the gut and its content were removed before the volume of the residual fluid within the gut was measured. Blood samples were then taken and, after the serum had been separated by centrifugation, the serum, the gut content, urine and sea water were analysed for chloride and freezing-point depression by the methods described previously (Morris, 1956).

Δ_t indicates the measured freezing-point depression. Δ_{Cl} indicates the freezing-point depression due to monovalent chloride, as given by the relation 293 mM./l. NaCl $\equiv$ 1° C.Δ (Krogh, 1939).

In order to determine the amount of sea water swallowed, an aliquot of the gut content was diluted to a known volume with water containing a little 0·1 N sodium hydroxide to develop the colour of the phenol red. The colour intensity of this solution was compared in a Duboscq colorimeter with that of a known solution in sea water diluted to almost the same colour intensity by a known volume of alkaline solution. In this way it is possible to determine the relative concentrations of phenol red and knowing the volume of residual fluid within the gut to calculate the amount of sea water swallowed and absorbed by the animal. As Smith points out, this method of measuring swallowed water would be open to question if phenol red were absorbed by the animal by way of the gut, gills or skin. Examination of the centrifuged plasma failed to reveal signs of phenol red even after the addition of alkali, nor could any be found in the tissues. The efficiency of the gut block was also checked in a few cases by placing animals which were known to have swallowed

sea water into 1 l. of fresh 50% sea water for some time. No phenol red could be detected in the water.

In order to assess the number of chloride excretory cells whole gill pouches were taken from each individual at the end of the experiments and were fixed in Helly's fluid. They were then post-chromed for 3 days at 37° C. to preserve phospholipid material (Baker, 1945) and were later embedded in paraffin wax. Sections four to five μ thick were cut from the outer surface of the gill pouch in a plane parallel to the long axis of the gill filaments. The first ten sections were discarded, and the two immediately following were used; one was stained by the Kull method for mitochondria (Baker, 1945), the other was coloured by the Sudan Black method for phospholipid as described by Thomas (1948). The chloride excretory cells are clearly distinguished by either method (Morris, 1957). The sections were examined using a one-sixth objective and no attempt was made to count the number of chloride excretory cells because of the difficulty of preparing strictly comparable sections owing to differences of curvature between individual gill pouches. Instead, representative sections from each animal were ranked in different categories according to the number of cells. Four groups were recognized: those containing 'many', 'few', 'very few' and 'nil'. There was no difficulty in distinguishing sections belonging to the extreme groups, only those in the groups 'few' and 'very few' proved difficult to place. Because of this, sections from both of these groups, stained by the same method, were ranked in order of the relative abundance of chloride excretory cells. The rankings were carried out quite independently on the two sets of slides and gave a 'coefficient of correspondence' (Moroney, 1951) of 0·72 indicating a good agreement between separate rankings. Separation into the two groups was then carried out on the ranked slides.

The experiments relating to gut diameter and swallowing capacity were conducted on maturing animals during the same season. Swallowing capacity in 50% sea water was determined by the method described above; gut diameter was measured in the pancreatic region of the gut (Barrington, 1944), since it is here, at the junction of the oesophageal and the intestinal regions, that the gut seems to become occluded as the animal matures. The animals were dissected to reveal the oesophagus, after which this was injected with a solution of warm, coloured gelatine. The solution passed backward into the pancreatic region of the gut and eventually flowed out through the cut end of the intestine. The pancreatic region was then dissected out, fixed and dehydrated in alcohol, after which it was cleared and mounted in cedar wood oil. The maximum and minimum diameter of the coloured gelatine plug was then measured by means of a microscope equipped with a micrometer eyepiece.

RESULTS

The results of eighteen successful experiments are recorded in Table 1. Experiments have been ranked according to the level of the plasma freezing-point depression (Δ_f) of the animals and are numbered in this order. Examples of typical responses have been selected and these are summarized in histogram form in Fig. 1.

Table 1. *The osmotic responses of lamperns immersed in 50% sea water*

Exp. no.	Initial weight (g.)	% change of wt. in 50% sea water	Environment and body fluids	Δ_t (° C.)	Calcd. Δ_{01} (° C.)	% $\frac{\Delta_{01}}{\Delta_t}$	Collection period (hr.)	Amount of fluid (ml.)	Fluid content of gut (ml.)			Chloride excretory cell assessment
									Intake	Absorbed	Residue	
1	70·8	+4·0	Sea water	0·925	0·820	88·8	19·5	2·1	8·4	6·3	2·1	Many
			Gut fluid	0·795	0·415	52·0						
			Plasma	0·565	0·390	69·0						
			Urine	—	—	—	6·5	Nil				
2	74·0	+3·0	Sea water	0·975	0·815	83·5	19·5	3·0	13·2	10·2	3·0	Many
			Gut fluid	0·580	0·425	73·0						
			Plasma	0·570	0·415	73·0						
			Urine	—	—	—	6·5	Nil				
3	79·6	+2·5	Sea water	1·00	0·885	88·5	9·0	1·5	6·0	4·5	1·5	Many
			Gut fluid	0·580	0·400	69·0						
			Plasma	0·575	0·400	69·5						
			Urine	—	—	—	9·0	Nil				
4	75·1	+2·5	Sea water	0·990	0·850	85·8	23·0	1·8	7·6	5·8	1·8	Many
			Gut fluid	0·585	0·465	79·5						
			Plasma	0·580	0·400	68·9						
			Urine	—	—	—	6·5	0·025				
5	64·1	−10·5	Sea water	0·965	0·820	84·9	20·5	0·08	0·18	0·10	0·08	Many
			Gut fluid	—	—	—						
			Plasma	0·660	0·450	68·1						
6	72·8	−10·0	Sea water	0·980	0·820	83·6	27·0	0·38	1·9	1·52	0·38	Few
			Gut fluid	—	0·615	—						
			Plasma	0·680	0·510	75·0						
7	80·65	−6·3	Sea water	0·925	0·820	88·6	18·5	0·10	0·33	0·23	0·10	Few
			Gut fluid	—	—	—						
			Plasma	0·740	0·510	68·4						
8	73·0	−10·9	Sea water	0·985	0·835	84·7	24·0	1·38	5·08	3·70	1·38	Few
			Gut fluid	0·700	0·520	74·2						
			Plasma	0·740	0·530	71·6						
9	50·0	−8·4	Sea water	0·925	0·820	88·6	19·0	Nil	Nil	Nil	Nil	Very few
			Gut fluid	—	—	—						
			Plasma	0·755	0·545	72·0						

Table 1 (*cont.*)

Exp. no.	Initial weight (g.)	% change of wt. in 50 % sea water	Environment and body fluids	Δ_t (° C.)	Calcd. Δ_{Cl} (° C.)	% $\frac{\Delta_{Cl}}{\Delta_t}$	Collection period (hr.)	Amount of fluid (ml.)	Fluid content of gut (ml.)			Chloride excretory cell assessment
									Intake	Absorbed	Residue	
10	100·2	−7·9	Sea water	0·980	0·835	84·5	27·0	2·08	9·5	7·42	2·08	Few
			Gut fluid	0·730	0·565	77·3						
			Plasma	0·770	0·585	75·9						
11	90·65	−4·1	Sea water	0·975	0·815	83·5	19·5	0·3	3·7	3·4	0·3	Few
			Gut fluid	—	0·440	—						
			Plasma	0·785	0·590	75·1						
12	76·7	−9·3	Sea water	0·970	0·825	85·0	23·0	0·40	0·4	—	0·4	Nil
			Gut fluid	—	0·650	—						
			Plasma	0·800	0·610	76·2						
13	70·5	—	Sea water	1·00	0·885	88·5	9·0	1·2	1·6	0·4	1·2	Few
			Gut fluid	0·830	0·705	85·0						
			Plasma	0·810	0·585	72·2						
14	68·9	−12·9	Sea water	0·970	0·825	85·0	23·0	0·9	0·9	—	0·9	Nil
			Gut fluid	0·790	0·635	80·0						
			Plasma	0·825	0·590	71·5						
15	88·5	−12·45	Sea water	0·990	0·850	85·8	19·0	1·6	5·8	4·2	1·6	Few
			Gut fluid	0·780	0·615	78·8						
			Plasma	0·840	0·590	70·2						
			Urine	0·680	0·445	65·5	4·5	0·72				
16	81·7	−8·0	Sea water	0·985	0·835	84·5	23·0	0·8	2·5	1·7	0·8	Nil
			Gut fluid	0·820	0·650	79·2						
			Plasma	0·840	0·610	72·6						
17	78·0	−10·0	Sea water	0·970	0·825	85·0	23·0	0·4	2·2	1·8	0·4	Nil
			Gut fluid	—	0·635	—						
			Plasma	0·845	0·635	75·0						
18	82·4	−11·7	Sea water	0·980	0·820	83·6	23·0	0·90	4·4	3·3	0·9	Nil
			Gut fluid	0·615	0·440	71·5						
			Plasma	0·870	0·655	75·0						

The experimental results obtained for each animal are represented by a maximum number of five blocks. The first four blocks refer to various fluids, as follows: (i) (Dashes) sea water swallowed. (ii) (Broken cross-hatching) fluid absorbed from gut. (iii) (Dots) fluid remaining in gut. (iv) (Unbroken cross-hatching) blood. The value of 5·7 ml. of blood per 100 g. found by Welcker and quoted by Reichert & Brown (1909) has been used to calculate the blood volume of each animal from its initial weight.

In the above blocks, the areas give a direct measure of the amounts of substance involved since Δ_t is given by total height; Δ_{Cl} is given by the height of the shaded area; ml./24 hr. is given by the width.

Block (v) is in fact a line rather than a block, whose height represents the chloride excretory cell assessment.

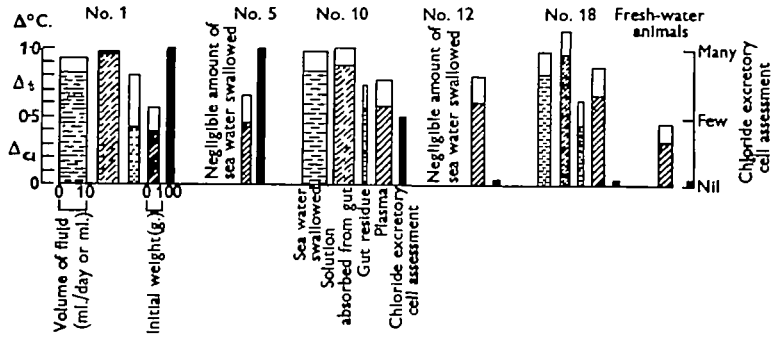

Fig. 1. The osmotic responses of selected fresh-run lamperns after immersion in 50% sea water (for further explanation see text).

THE CRITERIA OF SUCCESSFUL OSMOREGULATION

Two factors have been taken into consideration when deciding whether an animal has been osmoregulating successfully. These are (1) the ability to keep the body weight relatively constant, and (2) the ability to maintain the plasma osmotic pressure well below that of the environment. Only four animals satisfied these conditions in the present experiments. The mean values for freezing-point depression, blood chloride and percentage change of weight from the initial weight for these animals are recorded in Table 2. Similar measurements made on other fresh-run animals during an earlier series of experiments (Morris, 1956, fig. 7) are also included for comparison. These animals were immersed in 33% sea water ($\Delta_t = 0{\cdot}660°$ C.) for a minimum period of 48 hr.

As might be expected, the values for plasma freezing-point depression and chloride are somewhat lower in lamperns immersed in 33% sea water than those immersed in 50% sea water; the mean value of $\Delta = 0{\cdot}572°$ C. for the plasma of these atter animals compares favourably with the value recorded by Burian (1910) for the blood of related *Petromyzon marinus* ($\Delta = 0{\cdot}58°$ C.) living in sea water. This suggests that the lampern may also maintain its blood close to this concentration when living in sea water. The increase of plasma osmotic pressure (24%) brought about by

Table 2. *The plasma freezing-point depression* (Δ_f), *plasma chloride* (Δ_{Cl}) *and percentage change of weight from initial weight of fresh-run lamperns osmoregulating in various environments*

Environment	No. of observations	Plasma Δ_f (° C.)	Calculated plasma Δ_{Cl} (° C.)	% $\frac{\Delta_{Cl}}{\Delta_f}$	% change from initial weight
Fresh water	10	0·456 ± 0·006	0·325 ± 0·006	71·3	—
33 % sea water (Δ_f = 0·660° C.)	4	0·547 ± 0·011	0·359 ± 0·020	65·6	−2·5 ± 0·2
50 % sea water (Mean Δ_f = 0·970° C.)	4	0·572 ± 0·003	0·404 ± 0·005	70·6	+3·0 ± 0·3

Table 3. *Water balance in lamperns osmoregulating in* 50 % *sea water*

Exp. no.	Initial weight of animal, g.	Water balance (change in weight minus gut residue)		Water swallowed		Residual fluid in gut		Calculated water absorbed		% of swallowed water absorbed	Urinary output		Calculated extra-renal loss	
		g.	g./100 g./day	ml.	ml./100 g./day	ml.	ml./100 g./day	ml.	ml./100 g./day		ml.	ml./100 g./day	ml.	ml./100 g./day
1	70·8	+0·75	+1·3	8·4	14·7	2·1	3·7	6·3	11·0	75	Nil	—	5·55	9·7
2	74·0	−0·75	−1·3	13·2	22·1	3·0	5·1	10·2	17·0	77	Nil	—	10·95	18·3
3	79·6	+0·5	+1·8	6·0	20·1	1·5	5·0	4·5	15·1	75	Nil	—	4·0	13·3
4	75·1	+0·1	+0·14	7·6	10·5	1·8	2·4	5·8	8·1	77	0·025	0·13	5·6	7·83

transferring animals from fresh water to 50% sea water is of the same order as that recorded by Keys (1933) for the common eel (19%), when this animal is transferred from fresh water to full-strength sea water.

THE MECHANISM OF MARINE OSMOREGULATION

Water balance

Table 3 summarizes the data relating to water balance in the four osmoregulating animals. All of these results have been calculated in the following way. During Exp. 4, the lampern (initial weight, 75 g.) gained 1·9 g. over a period of 23 hr. immersion in 50% sea water. At the end of the experiment the gut contained 1·8 ml. of residual fluid to which most of the weight increase must be attributable. The condition of water balance at the end of the experiment must therefore have been +0·1 g. Measurements of the volume of residual fluid within the gut (1·8 ml.) and the relative concentration of phenol red showed that the animal had swallowed 7·6 ml. of sea water of which 5·8 ml. must have been absorbed. Since 0·1 g. of this contributed to the increase of weight and 0·1 ml. was excreted as urine, it follows that 5·6 ml. of water must have been lost by an extra-renal route, presumably by osmotic withdrawal through the permeable surface of the gills and, to a lesser extent, through the skin.

The rate of extra-renal loss of water is very much higher than that measured for the eel. The figures obtained by Smith (1930) give a loss of 6·25 ml. of water per 100 g. per day from an eel living in 100% sea water, an environment of twice the osmotic concentration of that in which the lamperns were kept. These findings confirm earlier comparisons of the relative permeabilities of the external surfaces of the two animals (Morris, 1956). In earlier experiments, as in the present ones, fresh-run lamperns showed a great deal of variability with regard to external surface permeability and, moreover, mature animals showed an increase in permeability when compared with fresh-run lamperns. It was, therefore, argued that permeability had already started to increase in fresh-run animals and that this process continued as the animals approached maturity. This possibly explains why the lampern is unable to osmoregulate in high concentrations of sea water even during the earliest stages of its spawning migration. The relatively high extra-renal loss may also account for the abnormally low urine outputs recorded for all osmoregulating animals. These values are lower than those given for marine teleosts which appear to vary between 0·2 and 0·5 ml./100 g. per day (Smith, 1930; Grafflint 1931; Pitts, 1933) and they may be taken as an indication of water shortage brought about by the abnormally high extra-renal loss.

Ion balance

Only chloride concentration and freezing-point depression were measured during the present investigation so that only anions can be considered. The data relating to anion balance are summarized in Tables 4 and 5 and they have been calculated in the following manner. Let us consider first the behaviour of chloride during Exp. 1.

Table 4. *Chloride balance in lamperns osmoregulating in* 50% *sea water*

Exp. no.	Cl swallowed		Cl in gut residue		Calculated Cl absorbed		% of swallowed Cl absorbed	Calculated amount and average concentration of Cl solution absorbed		Urinary Cl	Calculated extra-renal loss of Cl	
	mM.	mM./100 g./day	mM.	mM./100 g./day	mM.	mM./100 g./day		mM.	ml./100 g./day		mM.	mM./100 g./day
1	2·02	3·53	0·26	0·45	1·76	3·08	87	6·3 of 279	11·0 of 279	Nil	1·76	3·08
2	3·15	5·28	0·37	4·64	2·78	0·44	87	10·2 of 271	17·0 of 271	Nil	2·78	4·64
3	1·56	5·22	0·18	0·59	1·38	4·63	89	4·5 of 307	15·1 of 307	Nil	1·38	4·63
4	1·89	2·60	0·25	0·33	1·64	2·27	87	5·8 of 283	8·1 of 283	—	—	—

Table 5. *Balance of anions other than chloride* (*'A'*) *in lamperns osmoregulating in* 50% *sea water*

Exp. no.	'A' swallowed		'A' in gut residue		Calculated 'A' absorbed		% of 'A'	Calculated amount and average concentration of 'A' solution absorbed		Urinary 'A'
	mM.	mM./100 g /day	mM.	mM./100 g./day	mM.	mM./100 g./day		mM.	ml./100 g./day	
1	0·26	0·46	0·23	0·41	0·03	0·05	11	6·3 of 4·7	11·0 of 4·7	Nil
2	0·62	1·04	0·14	0·23	0·48	0·81	77	10·2 of 47·0	17·0 of 47·0	Nil
3	0·20	0·66	0·08	0·26	0·12	0·40	61	4·5 of 26·6	15·1 of 26·6	Nil
4	0·32	0·43	0·06	0·08	0·26	0·35	81	5·8 of 43·8	8·1 of 43·8	Nil

The animal swallowed 8·4 ml. of sea water during immersion in 50% sea water containing 240·0 mM. Cl./l., so that 2·02 mM Cl were taken into the gut. At the end of the experiment the gut residue amounted to 2·1 ml. and, since this contained 121·5 mM. Cl/l., the total amount of chloride in the gut residue becomes 0·26 mM. It follows from this that the animal absorbed 1·76 mM. Cl and, since this was absorbed at the same time as 6·3 ml. of fluid, the average strength of the chloride solution taken up becomes 279 mM./l. (Fig. 1). Because of the comparatively low level of blood chloride at the end of the experiment (144·5 mM./l.) it may be assumed that most of the chloride absorbed from the gut had been actively excreted by the animal into sea water containing roughly twice as much chloride as the blood and, since this particular animal produced no measurable quantity of urine, the excretion must have taken place by an extra-renal route.

Similar considerations apply to other osmoregulating animals, although in those cases where urine was produced it has been impossible to partition the excretion into renal and extra-renal routes because urinary chloride was not measured.

There is further evidence that chloride excretion takes place extra-renally. The osmoregulating mechanism was only partially effective in one of the animals studied (Table 1, Exp. 15) and here the lampern produced sufficient urine for it to be shown that the urine remained hypotonic to the blood. ($\Delta_{plasma} = 0{\cdot}840°$ C., $\Delta_{urine} = 0{\cdot}680°$ C.). The urinary chloride (130 mM./l.) was also maintained at a lower level than that of the blood (173·5 mM./l.) although the lampern was in obvious osmotic difficulties. This may be taken as an indication that the lampern kidney is similar to the kidneys of other lower vertebrates (Smith, 1932) and is unable to produce a hypertonic urine and conserve water in this way. The kidney is presumably orientated toward a fresh-water existence and thus continues to take up ions in spite of changed osmotic circumstances.

It is also possible to calculate the balance of non-chloride anions from the data available (Table 5 and Fig. 1, non-shaded areas). In sea water and the residual fluid of the gut these anions are presumed to consist mainly of sulphate, together with a little bicarbonate. The following method of calculation has been employed, using Exp. 1 again as an example. The freezing-point depression of 50% sea water in this case is equivalent to a total ionic concentration of 271 mM. NaCl/l. and, since the animal swallowed 8·4 ml. of sea water, this volume contained the equivalent of 2·28 mM. NaCl in total. 2·02 mM. of the anions are known to be chloride, so that the quantity of non-chloride anion swallowed was equivalent to 0·26 mM. NaCl. The same method of calculation can be applied to the gut residue (2·1 ml.: $\Delta = 0{\cdot}795°$ C.) and here the non-chloride anion amounts to 0·23 mM. NaCl. The animal must therefore have absorbed 0·03 mM. of non-chloride anion, which represents about 11% of the quantity originally swallowed by the animal. Since the non-chloride anion was absorbed with 6·3 ml. of fluid, the average concentration of fluid absorbed is equivalent to 4·7 mM. NaCl.

The general pattern of the various factors involved during absorption from the gut can be seen by reference to Fig. 1 and Table 1. Considering chloride first, it is noticeable that in osmoregulating animals the concentration of chloride in the

gut residue never drops below that of the blood. This suggests that the chloride ion enters the body fluids by a process of physical diffusion and, because the average concentration of chloride absorbed is somewhat higher than that of sea water which is swallowed, the chloride must enter faster than the water which accompanies it. Absorption of non-chloride fraction of the swallowed sea water could also be explained in the same way, since the inorganic anions (mainly sulphate) contributing to this fraction of the gut residue are certain to be in greater concentration than in the blood, which contains a relatively high proportion of organic materials acting as anions (Robertson, 1954). The rate of entry of non-chloride anion, however, varies between different individuals (Tables 1, 4). In Exp. 1 it enters at a much slower rate than either chloride or water and hence accumulates in the gut. The conditions in Exps. 3 and 2 are similar, although the rate of entry is much faster in these animals. In the last of the osmoregulating animals (Exp. 4) the concentration of non-chloride anion in the gut residue ($\Delta_A = 0{\cdot}120°$ C.) is less than that of the swallowed sea water and must therefore enter faster than the water absorbed at the same time. If, as seems likely, the processes of anionic absorption in the lampern gut are brought about by diffusion, then it may be assumed that these substances are either exchanged for other anions, or that cations accompany them. Judging from Smith's work (1930) on marine teleosts the latter process seems much more likely, since in these creatures monovalent ions (Na^+ and Cl^-) are absorbed preferentially, thus leaving the divalent ions (Ca^{2+}, Mg^{2+} and SO_4^{2-}) behind. The data presented for Exp. 1 suggest that a similar process takes place in the lampern and, since other animals show an increasing tendency to take up divalent anions, it may be that gut permeability becomes altered in some way during the migration.

The fate of the divalent ions taken up by these animals is uncertain. It is unlikely that they remain in the blood stream because the proportion of chloride to total ions remains almost the same in animals osmoregulating in 50% sea water as in fresh-run animals taken from fresh water (Table 2). It therefore follows that the proportion of non-chloride anions must also remain at the same level and, because these substances are continually entering the blood from the gut, they must be excreted in some way. Of the two possible pathways available for excretion, the renal route seems more likely than the extra-renal one because of the evidence derived from teleost material (Smith, 1930). In these animals, Ca^{2+}, Mg^{2+} and SO_4^{2-} ions which are taken up from the gut appear to be excreted in the urine. In the present experiments the quantity of urine produced was negligible, so that the non-chloride anions must either have been removed by the kidney and stored in any urine which may have been produced or they may have been extra-renally excreted.

THE BREAKDOWN OF MARINE OSMOREGULATION

An examination of Table 1 and Fig. 1 shows that the marine osmoregulatory powers of many fresh-run lamperns were failing. This failure can be attributed to changes in three of the main components of the marine osmoregulatory mechanism. The first two factors to be considered mainly affect the water balance of the animals.

Increase of the water permeability of the external surfaces

It has been argued already that an increase of water permeability may have taken place in animals which were found to be capable of osmoregulating in 50% sea water, and that this factor may be responsible in part for the breakdown of marine osmoregulation. Unfortunately, it has not been possible to assess water permeability in non-osmoregulating animals, so that this variable has had to be neglected in the analysis which follows.

The decrease in swallowing capacity

The problem of comparing the osmoregulatory ability of non-osmoregulating animals in terms of plasma freezing-point depression is complicated by unavoidable variations in the concentration of the external environment (Table 1). In osmoregulating animals it has been shown that an increase of environmental osmotic pressure from 33% sea water ($\Delta_f = 0{\cdot}660°$ C.) to 50% sea water (mean $\Delta_f = 0{\cdot}970°$ C.) brings about an increase of plasma freezing-point depression of 0·025° C. (Table 2). It thus seems that animals which are osmoregulating or partially osmoregulating are unlikely to be affected significantly by small variations in the external environment. In the series of animals showing lower plasma freezing-point depressions, small variations in sea-water concentration might be expected to have progressively more significant effects on plasma freezing-point depression as the animals become less independent of their environment. Even in the worst of these cases, however, the variation of the plasma must always be less than that of the environment, because the animals never reach isotonicity during the period of the experiments. For these reasons, variations in the concentration of the external environment have been neglected and osmoregulatory ability has been assumed to be directly related to plasma freezing-point depression.

The relationship between swallowing capacity and osmoregulatory ability (Fig. 2) is a complex one, because the plasma osmotic pressure also varies with the amount of extra-renal excretion which takes place at the same time. It is, therefore, only possible to consider the relationship in those cases where the extra-renal excretory powers can be assessed, that is, in those animals in which the number of chloride excretory cells could be classed either as 'many' or as 'absent'. In those animals containing the maximum number of excretory cells (Fig. 2, black triangles), the osmoregulatory ability decreases as the rate of swallowing decreases, and in osmoregulating animals the differences in swallowing rates are an expression of the effect of the differing water permeabilities of their external surfaces (Table 3). The animals without chloride excretory cells (open circles) show an increase in plasma osmotic pressure with an increased rate of swallowing. The explanation of this relationship is probably that the gut, being permeable to ions, allows these substances to enter the blood stream and, since the extra-renal excretory mechanism is lacking, the plasma osmotic pressure rises in proportion to the amount of water swallowed.

Although the cause of the reduction of swallowing capacity is uncertain, it is thought to be connected with degeneration of the alimentary tract. It is well known that in both the lampern and the sea lamprey the gut starts to atrophy when the animal commences its spawning migration (Keibel, 1927; Applegate, 1950). During the present studies, measurements of the gut diameter of maturing animals (March and April) showed that there was a great deal of variation in the pancreatic region, from almost complete closure (0·3 mm.) to maximum diameters of 2·3 mm. Some

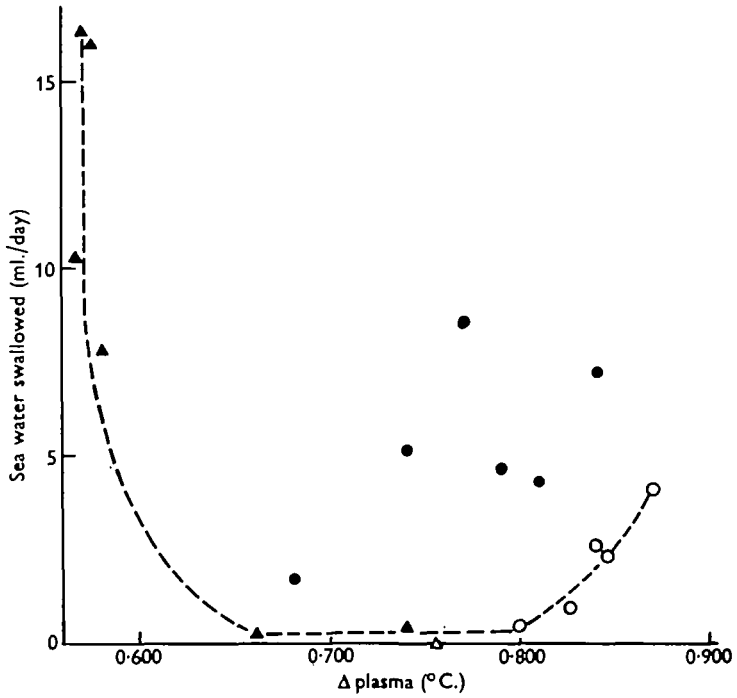

Fig. 2. The relationship between swallowing capacity, chloride excretory cell assessment and plasma freezing-point depression (Δ) in fresh-run lamperns immersed in 50% sea water. Chloride excretory cell assessment: ▲, many; ●, few; △, very few; ○, nil.

of these animals still swallowed a little sea water when immersed in 50% sea water, but there was only a weak correlation between cross-sectional area of the gut and swallowing capacity. This could have been due to the difficulties of assessing the cross-sectional area of the gut which, in these experiments, was only calculated approximately from the maximum and minimum diameters of the gut at its narrowest point, assuming an ellipsoid cross-section.

Decrease in extra-renal excretion

If those animals are considered which do not swallow sea water (Fig. 2), there appears to be a relationship between chloride excretory cell assessment and osmoregulatory capacity of the type which might be expected if extra-renal excretion was

diminishing in some individuals. The pattern of behaviour of the animals containing 'few' excretory cells (black circles) is much more difficult to interpret, because these show the widest spread of variation in numbers of excretory cells, in osmotic response and in swallowing capacity. On the whole, however, they appear to support the general conclusions that a reduction in swallowing rate and/or a decrease in chloride excretory cell numbers results in a loss of osmoregulatory capacity.

DISCUSSION

Considered within the limits set by the present analysis, the marine mechanism of osmoregulation in the lamperns appears to be strikingly similar to that employed by marine teleosts (Smith, 1930; Keys, 1931*a*, 1931*b*, 1933). In these animals, the water and salts from swallowed sea water are absorbed selectively from a region of high concentration within the gut to one of lower concentration in the blood. Monovalent ions are absorbed faster than water and the divalent ions which remain in the gut are eliminated in the rectal fluid. Almost all of the excess monovalent ions are excreted extra-renally by tissues located in the gills which are capable of performing osmotic work, whilst the excess divalent ions which are absorbed are excreted in the scanty, but hypotonic urine. The end result of these processes is the continuous replacement of body water which is lost as a consequence of both the osmotic situation and the formation of urine. Apart from the renal elimination of divalent ions, where doubt remains because of the difficulties of analysis, the mechanism in the osmoregulating lamperns appears to follow the same pattern. The main point of difference between the lampern and the marine teleosts seems to be that the external surfaces of the former are very much more permeable to water. There may be at least two reasons why water permeability is very high in fresh-run lamperns. Bahr (1952) has shown that even newly metamorphosed and sexually immature adult lamperns begin to suffer in concentrations above 65% sea water and this, together with field observations, has led him to believe that the majority of adult lamperns spend their life in the reduced salinity found in estuarine waters. There is therefore little reason to expect most lamperns to develop the high resistance to osmotic loss which is so evident in marine teleosts. In addition to this, there is also evidence that the water permeability of the lampern changes during the course of its spawning migration. A comparison of the weight changes of fresh-run and maturing lamperns during the early stages of experiments involving immersion in dilute sea water, indicates that the water permeability of the external surfaces increases as the animals mature (Morris, 1956). This corresponds with the observation of Hardisty (1956), that mature animals show an increase in water content. Earlier work (Morris, 1956) also stressed the variability in water permeability in fresh-run animals and this is certainly borne out by the variable nature of extra-renal loss of water in the present experiments (Table 2). This variation may be the result of increased water permeability which would be expected to take place at different rates in some individuals at the beginning of the spawning migration. The low urine output recorded for osmoregulating animals indicates that they are

suffering from water shortage and this also tends to support the view that the external surfaces have increased their permeability to water.

The observations on non-osmoregulating animals also confirm that the mechanism is teleost-like, since they give the opportunity of studying the effects of removal of parts of the mechanism without having to interfere with the animals surgically. Animals which have lost either the swallowing mechanism or the extra-renal excretory mechanism behave in ways which might be expected from a knowledge of the complete mechanism and thus confirm the osmoregulatory function of these processes. The relationship between osmoregulating ability and chloride excretory cell assessment is particularly important in view of the doubts expressed by Bevelander (1935, 1936) and Krogh (1939) concerning the excretory function of these cells. The present studies, together with those of Keys & Willmer (1932), Keys (1931*a*, 1931*b*), Copeland (1948), Liu (1942, 1944), Getman (1950) and Morris (1957), establish beyond reasonable doubt the excretory function of these structures in both the lampern and in marine teleosts.

Homer Smith has argued that the teleosts owe their peculiar marine osmoregulatory mechanism to their fresh-water ancestry. According to the view of Marshall & Smith (1930), the vertebrate kidney was developed originally as a means of dealing with the osmotic intake of water from this environment and, in consequence, its function was orientated toward water excretion and ion retention. When the teleosts invaded the seas, little change took place in kidney function initially in order to cope with changed osmotic circumstances, and the animals continued to produce a urine which was hypotonic to their blood, although diminished in quantity and augmented in ion content. This was made possible by the development of the swallowing habit combined with the extra-renal excretory mechanism described above (Smith, 1932, 1953). The ideas of Marshall and of Smith on the fresh-water origin of the vertebrate kidney have been questioned recently by Robertson (1954, 1957). Robertson finds that the earliest vertebrates were marine and argues for a marine origin of the glomerulus, since glomerular development does not appear to be proportional to the osmotic gradient in groups other than the Osteichthyes. These views do not, however, appear to conflict with Smith's hypothesis concerning the marine osmoregulating mechanism of the teleosts and there seems little doubt about the fresh-water origin of this group. Because of the remote relationship between lampreys and teleosts, the development of similar marine osmoregulatory mechanisms must have taken place independently and this could be attributed to parallel evolution brought about by similar osmotic circumstances during the past history of the two groups. It thus seems probable that the lampreys also developed in fresh water—a view which is strengthened by a consideration of their life history and migratory habits.

SUMMARY

1. Some fresh-run lamperns can osmoregulate in 50% sea water ($\Delta = 0{\cdot}970°$ C.), where they can maintain plasma freezing-point depressions of about 0·57° C.

2. An analysis of the mechanism of osmoregulation in these animals shows that it is similar in many respects to that employed by marine teleosts. The lampern swallows sea water and absorbs a solution containing a high proportion of monovalent ions into its blood. It has been calculated that chloride is excreted by an extra-renal route, presumably by means of chloride excretory cells which have been discovered in the gills. The rate of extra-renal loss of water is high and the urine output is negligible.

3. Many fresh-run animals are unable to osmoregulate. In some cases the capacity to swallow sea water becomes reduced, whilst in others the number of chloride excretory cells is diminished—an indication that the extra-renal excretory mechanism is failing. Both of these mechanisms regress simultaneously in some animals.

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