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SODIUM BALANCE IN THE EGGS OF THE ATLANTIC SALMON, *SALMO SALAR*

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INTRODUCTION

Although the eggs of salmon and trout have long been of interest to physiologists the extent to which the eggs are permeable to sodium ions is not clear from previous accounts. Freshly stripped salmon eggs contain 2–3 μ-equiv. Na/egg. During embryonic development the quantity of sodium in the egg remains approximately constant (Hayes, 1949), but sodium balance in the freshly stripped egg is complicated. From experiments with freshly stripped eggs in hyperosmotic saline, containing 0·55 M-NaCl/l., Kalman (1959) inferred that some sodium penetrated into the yolk, but nevertheless the sodium concentration in the yolk remained much lower than in the medium. Parry Howells (personal communication) has reported that the perivitelline fluid concentrates sodium ions in the water-hardened egg. In order to clarify the situation the exchange of sodium has been examined in freshly stripped, water-hardened eggs, and in eggs in the eyed stage.

MATERIALS AND METHODS

Salmon were obtained from the River Lune at Broad Raine. Eggs were stripped into plastic bowls and maintained at 10° C. Total sodium was estimated by flame photometry using an EEL flame photometer, after digesting the eggs in concentrated nitric acid. Flux measurements were made using ^{24}Na and an EKCO sodium iodide well-crystal counter. For counting, 10 eggs were removed from the medium, dried thoroughly on tissue paper and counted in a test tube. The media used were isotonic saline (150 mM-NaCl/l.) containing ^{24}Na and river water to which ^{24}NaCl was added to make an over-all concentration of sodium of approximately 1 mM/l.

RESULTS

Sodium content of eggs

Eggs from different fish showed some variation in sodium content. Most experiments recorded here were carried out on a batch of eggs that contained 2·2 μ-equiv. Na/egg or 20 μM/g. egg. At 50 days, shortly before hatching, this had increased to 2·6 μ-equiv./egg. On hatching there was a small loss of sodium associated with the loss of the chorion and perivitelline fluid, but sodium uptake continued. The results are summarized in Table 1.

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Eggs in saline

When freshly stripped eggs were transferred to 150 mM-NaCl/l., the activity in the eggs increased rapidly, reaching equilibrium after about 15 min. At this point the specific activity of the eggs was about 20% of that of the loading solution, but the sodium concentration of the medium was more than seven times that of the original egg. Hence the activity can be accounted for if 3% of the egg volume equilibrated with the medium. No difference was observed between fertilized and unfertilized eggs.

Table 1

Age	Weight (g.)	Na content (μ-equiv./egg or alevin)
Freshly stripped	0·0866	2·2
1 hr.	0·1004	2·2
2 hr.	0·1027	2·2
40 days	0·1272	2·6
1-day alevin	0·1030	3·5
10-day alevin	0·1076	3·8

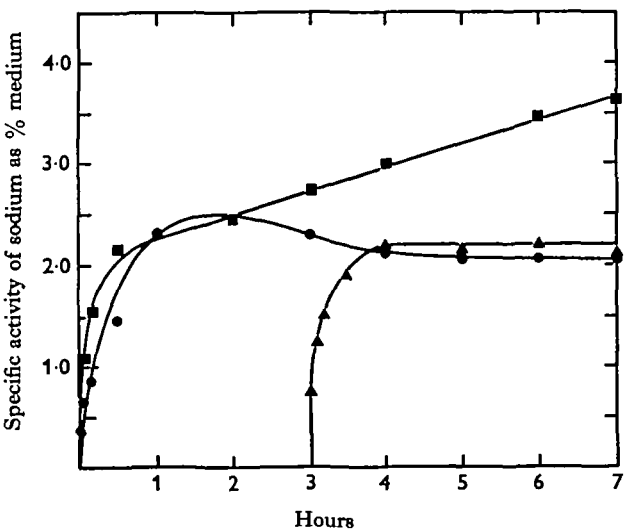

Fig. 1. Changes in specific activity of the eggs of *S. salar* when placed in river water containing 1 mM-NaCl/l. labelled with ^{24}Na. ●, Freshly stripped; ▲, water hardened; ■, eyed.

Sodium exchange in river water

When freshly stripped eggs were placed in river water containing ^{24}Na the activity in the egg increased for about 1½ hr. concomitant with the formation of the perivitelline fluid, and then declined slightly. At the end of this period the specific activity in the whole egg was more than 2% of that of the medium. No further increase in activity occurred during the next 7 hr. (Fig. 1). When eggs that had been water-hardened for 3 hr. were placed in river water the activity increased more rapidly and reached equilibrium in about 30 min. (Fig. 2). Once again the specific activity of the egg was about 2% that of the medium. If the activity inside the egg were at the same concentra-

tion as outside (1 μM/ml.) the accessible space of the egg would have to be 50% of the total volume or over 80% of the water content. This is considerably greater than the volume of the perivitelline fluid together with the chorion. Alternatively, if the activity were confined to the chorion and perivitelline fluid, which comprise 17% of the egg volume, the sodium concentration would have to be 3 mM/l. in these fluids. Once again there was no difference between fertilized and unfertilized eggs. When transferred to inactive solutions the activity was rapidly and almost completely lost.

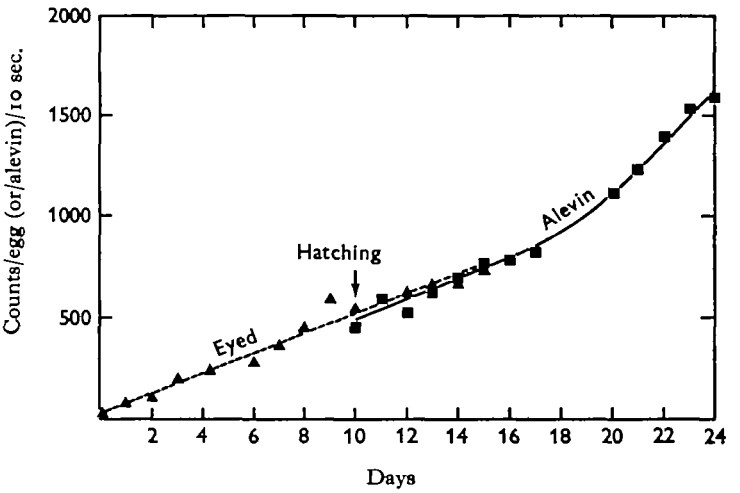

Fig. 2. Increase in activity of late eggs and alevins of *S. salar* when placed in river water containing 1 mM-NaCl/l. labelled with ^{24}Na. ▲, eyed; ■, alevin.

Sodium exchange in water-hardened eggs in saline

In 2-week-old water-hardened eggs the accessible sodium space was equivalent to 15% of the egg volume, or 23 μ-equiv./g. egg. This is very similar to the volume of the perivitelline fluid and chorion together—17% in fresh water-hardened eggs during the first day (Potts & Rudy, 1968).

Sodium exchange in the perivitelline fluid

Although there is considerable exchange of sodium in eggs in saline this may be attributed to a limited volume of water, most probably in the chorion and perivitelline fluid, which exchanges freely with the environment. Samples of yolk from eggs loaded in solutions containing ^{24}Na usually contained no activity. Occasional low activity found may be attributed to contamination of the yolk with perivitelline fluid, which is difficult to avoid. In dilute solutions the total exchangeable sodium is small but is equivalent to a sodium space of up to 150% of the egg volume. This implies that some sodium is concentrated either in the yolk or in the perivitelline fluid or chorion.

Pure samples of perivitelline fluid are difficult to collect but contamination with yolk when it occurs is obvious. When the external concentration was low, yolk-free samples of perivitelline fluid always contained a greater concentration of activity than the loading solution in which the eggs had been placed (Table 2). Perivitelline fluid concentrated sodium from the medium; the concentration of sodium in the perivitelline

fluid varied with the concentration in the medium but the concentration ratio varied inversely with the concentration in the medium.

The collection of perivitelline fluid is difficult and tedious. The sodium-holding properties of the perivitelline fluid were therefore determined using whole eggs on the assumptions that in high concentrations of sodium the concentration ratio of perivitelline fluid to medium was unity (as in Table 2) and that the volume of the water-hardened egg accessible to sodium was constant. The resulting estimates of sodium concentration agree well with the direct measurements (Table 3). The perivitelline fluid evidently contains some indiffusible anions which accumulate sodium above the

Table 2. *Concentration of sodium in perivitelline fluid of eggs of the Atlantic salmon,* Salmo salar

Concentration of media (mM-NaCl/kg.)	Concentration of Na in perivitelline fluid (mM/kg.)		
	Mean	Range	*N*
150	148	145–151	3
10	20·1	17·5–23·6	3
1	5·0	4·0– 7·8	6

Table 3. *Concentration of sodium in the perivitelline fluid (PVF) of the eggs of the Atlantic salmon,* S. salar, *in various concentrations of sodium chloride*

Media (mM-NaCl/l.)	Accessible egg volume (%)*	Concentration ratio in PVF†	Estimated concentration in PVF (mM-NaCl/l.)
150	15·2	1	150
100	15·4	1·02	102
10	1·7	1·3	31
10	26	1·7	17
3	37·9	2·5	7·5
1	76·5	5·05	5·05
0·3	96·1	6·3	1·9
0·1	145	9·5	0·95

* Calculated on the assumption that the concentration of sodium in the accessible space in the egg is the same as the medium.

† Calculated on the assumption that sodium exchange is confined to 15·2 % of the egg volume and that the concentration ratio is unity in the most concentrated medium.

environmental concentration to a maximum of about 10 mM/kg. It should be noted that although the concentration in 150 mM/l. saline is identical on a volume basis the perivitelline fluid will be more concentrated than the external medium on a water-content basis. The perivitelline fluid is a viscous fluid. If it contained for example 3 % solid matter the sodium concentration in the perivitelline fluid would be as high as 155 mM/kg. water in the most concentrated medium.

Osmotic concentration of perivitelline fluid

Measurements of the osmotic concentration of the perivitelline fluid in eggs from river water gave a mean osmotic concentration of 31 ± 3 m-osmoles/l ($n = 8$). The osmotic concentration of the Lune water was 2 m-osmoles and the sodium concentration was 0·23 mM/l. The sodium content of the perivitelline fluid water under these conditions should be less than 2 mM/l.

Sodium uptake in late embryos

The total sodium content of the eggs and the associated changes on hatching are generally similar to those reported by Hayes, Darcy & Sullivan (1946). However, it is clear that sodium accumulation starts some time before hatching. In the eyed stage, shortly before hatching, the egg no longer equilibrates with sodium in the external medium but accumulates sodium at a rate of about 0·3 %/hr. (Fig. 1). It is not known when sodium accumulation begins. The increase in sodium between stripping and hatching amounts to almost 20 %. This is equivalent to barely 2 days' accumulation (Table 1). This suggests that accumulation is accompanied by almost equivalent losses.

DISCUSSION

These experiments suggest that in freshly stripped living eggs in isotonic saline or in fresh water, sodium exchange is confined to the chorion and the perivitelline fluid, although in the eyed stage some active uptake of sodium into the embryo takes place. This is in agreement with the results of Hayes *et al.* (1946) who found that the total sodium content of the egg plus developing embryo of *Salmo salar* remained constant during the first half of the development period. In contrast, Kalman (1959) claimed that the vitelline membrane of trout eggs (*S. gairdneri*) is permeable to sodium ions, but his arguments are obscure. His experiments were carried out in a strongly hyperosmotic saline containing 550 mM-NaCl/l. In this medium water-hardened unfertilized eggs were described as exchanging 30 % of the egg water (Kalman, table 1) and 19 % of the sodium (Kalman, p. 157) in 15 min., by which time they had reached equilibrium. Eggs that had been heated for 3 min. (and probably killed) exchange 62 % of the water and '68 % of the sodium'. Eggs in fresh water contained only 15 m-equiv.-Na/kg. In such a concentrated solution, 550 mM/l., the sodium content of the perivitelline fluid and chorion would be many times greater than the sodium content of the yolk and it seems likely that the 19 % and 68 %, etc. refer to the accessible sodium spaces rather than to the fraction of sodium exchanged. In a further series of experiments the proportions equilibrated were: whole egg, water 30 %, sodium 27 %; egg-yolk water 13·8 %, egg-yolk sodium 9·5 %. The reason for the difference between the two experiments is not explained and the derivation of the yolk figures are not given; but later in the paper Kalman calculates the sodium exchange in the yolk on the assumption that perivitelline fluid (+ chorion) is equivalent to only 10 % of the total egg water. If the perivitelline fluid was underestimated, and 10 % is very low in view of the amount of water that equilibrates in 15 min., this would overestimate the exchange in the yolk. However, even if one accepts Kalman's conclusions it should be noted that the solutions used contained an even higher concentration of sodium than the sea water. In fresh water any sodium contribution to the egg yolk would be negligible. It is clear from Kalman's experiments that the vitelline membrane of heat-treated eggs is permeable to sodium and more permeable to water than that of normal eggs.

The perivitelline fluid protects the delicate vitelline membrane and the developing embryo from damage and provides room for embryonic development. The formation of the perivitelline fluid is attributed by Bogucki (1930) to the release from the yolk into the perivitelline space of colloidal substances. Bogucki (1930) and Hayes (1949)

suggested that the colloids could not escape through the chorion but that they 'imbibed' water until the tension of the chorion balanced the imbibition pressure (?colloid osmotic pressure). In support of this theory it may be noted that the perivitelline fluid is viscous and positive to Millon's reagent (Svetlov, 1929). The chorion is permeable to water and to dyes such as neutral red, but impermeable to larger molecules such as starch or egg albumen (Hayes, 1949). The slight decline in the sodium content of the perivitelline fluid after 2–4 hr. in fresh water suggests that some organic material may be lost through the chorion. Svetlov (1929) reported that the perivitelline fluid was practically isosmotic with river water ($\Delta 0{\cdot}02^\circ$ C $\equiv$ < 10 mM/l.) but the present authors find that in Lune salmon at least, the osmotic pressure is about 30 m-osmoles/l. Some part of the osmotic pressure will be due to associated sodium and calcium ions but the greater part must be due to the organic colloid. A colloid concentration of *ca* 25 m-osmoles/l. is equivalent to a hydrostatic pressure of half an atmosphere. Kao & Chambers (1954) recorded a hydrostatic pressure of 150 mm. Hg in the eggs of *Fundulus heteroclitus*, equivalent to one-fifth of an atmosphere. A colloid osmotic pressure of half an atmosphere is unusually high for biological systems and probably implies a lower molecular weight than is found, for example, in mammalian blood plasma.

Most proteins have an excess of anions in a neutral solution and this would account for the ability of the perivitelline fluid to concentrate sodium ions, although the concentration ratios in Table 2 do not correspond with a Donnan equilibrium at the lower concentrations. For example, if the anion excess was about 10 m-equiv./kg. water then in a solution containing 150 mM-NaCl/l. the internal concentration would be 155 mM-Na/l. and 145 mM-Cl/l. In 10 mM-NaCl/l. the internal concentrations would be 16·2 mM-Na/l. and 6·2 mM-Cl/l., in fair agreement with experiment; but in 1 mM-NaCl/l. the internal concentrations should be 10·1 mM-Na/l. and 0·1 mM-Cl/l., whereas the observed sodium concentration is only half this value.

The agreement between theory and experiment would be increased if it were assumed that the chorion does not accumulate sodium above the ambient concentration. The accessible water space of the water-hardened egg in 150 mM-Na/l. will consist of both chorion water (3 % egg weight) and perivitelline fluid (13 % egg weight) but the concentration effect is probably confined to the perivitelline fluid. If the data are recalculated on the assumption that the concentration effect is confined to the perivitelline fluid the concentration ratios will be increased in the more dilute media by about 20%; but the concentration ratios will still be too low in dilute solutions. However, Donnan ratios are not maintained in any system under limiting conditions. As the external concentration approaches zero the concentration ratio, and the associated potential, would approach infinity by the classical Donnan theory. In practice, the increasing potential concentrates hydrogen ions along with the other cations and these associate with the weakly ionized acid radicals, thus reducing the anion excess which is the cause of the Donnan equilibrium.

In addition, the changing activity coefficients on the two sides of the membrane further reduce the effective concentration ratio as the activity coefficient in the more dilute solution increases more rapidly than in more concentrated solutions as the concentration declines.

The establishment of a Donnan equilibrium across the chorion probably accounts

for the ability of salmon eggs to take up sodium from the medium. The volume of fluid required, *ca* 16% of the egg volume, agrees well with the observed increase of weight on water-hardening together with the chorion water (17%) (Potts & Rudy, 1968). Similarly, the accessible space required to account for salt uptake in eggs shed into saline (3%) is equivalent to the water content of the chorion alone. (Note that in the swollen water-hardened eggs the chorion water is about 3·3% of the egg weight but in the smaller freshly stripped eggs it is 3·85%.) Freshly stripped eggs placed in fresh water do not accumulate sodium as rapidly as do water-hardened eggs. This confirms that the organic colloids which cause the Donnan equilibrium are not originally present outside the vitelline membrane but are liberated into the perivitelline space during the formation of the perivitelline fluid. They are probably the same substances as are responsible for the formation of the perivitelline fluid. The vitelline membrane at this stage is probably impermeable to sodium ions. In the eyed stage, however, active uptake of sodium begins, probably at the surface of the embryo. Thus osmoregulation begins before hatching. Hatching is associated with a small loss of sodium, probably that present in the perivitelline fluid and chorion, but after hatching the rate of salt uptake increases.

SUMMARY

1. Exchanges of sodium ions between the egg of the salmon and the environment have been examined at different stages.

2. In freshly stripped eggs and during the early stages of development exchange is confined to the chorion and perivitelline fluid.

3. The perivitelline fluid can accumulate sodium to several times the ambient concentration probably by a Donnan effect associated with its protein content. At low external concentrations the relative accumulation is lower than the Donnan theory predicts.

4. Sodium accumulation begins during the eyed stage and accelerates after hatching.

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Note added in proof. Klekowski & Domurat (1967) report that the perivitelline fluids of trout eggs in fresh water (*S. trutta*) are also initially hyperosmotic to the medium but decline to that of the medium shortly before hatching. The mean freezing point depression recorded, 0·030 °C is equivalent to 16 m osm/l.

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