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WATER BALANCE IN THE EGGS OF THE ATLANTIC SALMON *SALMO SALAR*

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INTRODUCTION

The osmotic and ionic properties of the eggs of trout and salmon have attracted the interest of physiologists for many years. The eggs are convenient biological material and immense numbers of large, almost identical eggs can readily be obtained. The very slow development of the eggs raises in an acute form the ionic and osmotic problems common to the eggs of all freshwater animals.

When shed into isotonic saline, eggs of salmon and trout maintain an almost constant weight and a relatively high permeability to water. When shed into fresh water the eggs become almost impermeable to water but increase in weight by about 16%, during the first 2 hr., as the result of the formation of a perivitelline fluid beneath the chorion (Bogucki, 1930). Eggs in this impermeable state are described as 'water-hardened'. The decline in permeability to water is due to a change in the properties of the vitelline membrane (Krogh & Ussing, 1937), the outer chorion remaining permeable to water even in the water-hardened condition (Gray, 1932). Krogh & Ussing (1937), as the result of experiments with deuterium oxide, decided that the permeability of the vitelline membrane became immeasurably low. More recently, Kalman (1959), using tritiated water, showed that there was a small exchange of water between the yolk and the surrounding media even in the water-hardened eggs of the trout, *Salmo gairdneri*, but no estimate of the permeability was made. The formation of the perivitelline fluid is inhibited in a saline solution but the perivitelline fluid is formed when the eggs are shed into isotonic urea or glucose solutions (Bogucki, 1930). In this paper we describe the uptake and exchange of water in the eggs of *Salmo salar* when shed into a variety of media.

METHODS

Eggs were obtained from salmon removed from the River Lune at Broad Raine, Westmorland, in November 1966 and November 1967. Eggs and milt were stripped into plastic bowls, previously rinsed in salmon Ringer. Every effort was taken to avoid contamination of the eggs with river water which could initiate 'water-hardening' but it is possible that during the stripping small amounts of river water were occasionally introduced. The majority of experiments were carried out with fertilized eggs, but for comparative purposes a few experiments were carried out with unfertilized eggs. The temperature of the river water at the time of the experiments was 3·5° C. The experi-

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ments were continued in a water-bath controlled at this temperature. A sample of River Lune water collected the previous year contained 0·45 mM-Ca/l. and 0·11 mM-Mg/l. The salmon Ringer was modified from that of Holmes & Stott (1960) and contained rather more calcium and less phosphate in order to bring the minor constituents more into line with the analysis of the coelomic fluid of *S. salar* by Hayes, Darcy & Sullivan (1946).* The glucose solution contained 320 mM glucose/l. Experiments were also carried out in a variety of concentrations of sodium and calcium ions (Fig. 4). All experiments with tritiated water were carried out in solutions containing 1 μC. of tritium per ml. of solution.

Freshly fertilized eggs are contaminated with considerable volumes of coelomic fluid and semen. At the beginning of experiments involving dilute solutions the eggs were first rinsed rapidly in a large volume of a similar, non-tritiated solution. The eggs were then transferred to a plastic vessel containing the experimental radioactive solution. Samples of fifty eggs were removed at intervals, blotted dry with tissue paper and transferred to weighed containers. Water was removed from the eggs for tritium assay by a freeze-drying process described previously (Rudy, 1967). Activity was measured with a Nuclear Enterprises Liquid Scintillation Counter using NS 220 scintillation fluid. Water contents were obtained by drying the eggs to constant weight at 100° C. Calcium and magnesium analyses were carried out by atomic absorption spectroscopy.

Calculation of the exchange constant of water within the vitelline membrane

It is not practicable to calculate the exact permeability of the vitelline membrane in absolute terms because the surface area of the vitelline membrane changes continuously during development and it is difficult to estimate this area exactly. Estimates of the diameter of the yolk sac made with the chorion intact would be subject to error which would be further compounded when calculating the area. It is difficult to remove the chorion from the egg without breaking the vitelline membrane. Instead, the rate constant of water exchange across the vitelline membrane has been calculated. To a first approximation this will be proportional to the permeability of the vitelline membrane.

After transfer to dilute solutions the eggs gain weight as the result of the formation of the perivitelline fluid. The water in the egg is distributed between the chorion, the perivitelline fluid and the yolk. In order to calculate the activity present in the yolk, the activity present in the chorion and in the perivitelline fluid must first be allowed for. From the increase in the weight of the egg, the quantity of water in the perivitelline space can be calculated. Determination of the water content of isolated chorions show that the water content of the chorion alone is 3·0 mg., equivalent to 6% of the total water content of freshly stripped eggs. Freshly stripped eggs, removed from the loading solution before any significant perivitelline fluid had been formed, had a specific activity of 5–10% that of the loading solution, probably corresponding to water in the

* The salmon Ringer was prepared by dissolving the following constituents in 1 l.: sodium chloride 8·5 g.: potassium chloride 0·4 g.: calcium chloride 0·5 g.: disodium hydrogen phosphate 0·2 g.: sodium dihydrogen phosphate 0·05 g.: sodium bicarbonate 0·1 g.: and magnesium sulphate 0·15 g. The final concentrations were 150 m-equiv. sodium/l., 151·9 m-equiv. chloride/l., 5·4 m-equiv. potassium/l., 4·5 m-equiv. calcium/l., 1·25 m-equiv magnesium/l., 1·25 m-equiv. sulphate/l. and 2·0 m-equiv. phosphate/l. The total osmotic concentration was 320 m-osmole/l.

chorion, together with any pre-existing perivitelline fluid and surface contamination. Since the perivitelline fluid is formed by the uptake of water from the external media, and since when fully formed it continues to exchange both water and sodium rapidly with the external medium (see below) it may be assumed that the specific activity of the water in both the chorion and the perivitelline fluid approximates to that of the medium. By using the following formula the specific activity of the water within the yolk can be calculated. If A is the specific activity of the loading solution, W the initial water content of the egg in mg., excluding the chorion, w the increase in the weight of the egg in mg., B the specific activity of the yolk water, then

$$\text{mean specific activity of egg water} = \frac{3A + wA + WB}{3 + W + w}.$$

It assumed that the water content of the yolk does not increase significantly during the experiment. Calculations showed that net water influx into the yolk will be negligible during the first few hours. In the period immediately following the transfer to the radioactive solution by far the greater part of the activity will be confined to the chorion and the perivitelline fluid. Any error in the allowance made for these two compartments will therefore produce a relatively large error in the calculated specific activity of the yolk. This error will be largest in the first time interval when the specific activity of the yolk is very low. However, estimates of egg weights are very precise. The average weights of single eggs from the three fishes used in these experiments were 86·8., 93·6 and 83·9 mg., but the variation amongst eggs from individual fish was very small, *ca.* 1%. Similarly, in the trout, *Salmo gairdneri*, the average variation from the mean weight between eggs from different fishes was 10% but the average variation from the mean in eggs from a single fish was only 0·5% (Manery & Irving, 1934).

From the change in the specific activity of the water in the yolk the exchange constant for yolk water can be calculated for each time interval from the equation

$$K = \frac{1}{T} \log_e \frac{A - B_1}{A - B_2},$$

where B_1 and B_2 are the specific activity of the yolk on two occasions at time T apart. K is the fraction of yolk water exchanging per unit time. Once again any errors are compounded in calculating K. The osmotic flux will be only a small fraction of the gross flux. K will be proportional to the permeability of the vitelline membrane for yolk of constant volume.

RESULTS

Each experiment was confined to eggs from a single fish. Figure 1 shows the change in water content of unfertilized eggs from one particular fish after stripping into river water. The initial mean weight of the eggs was 93·6 mg. Rapid water uptake was completed in the first 3 hr. This was followed by a very slow increase in the weight of the egg, due mainly to water entering the yolk. The chorion water remained constant and there was a slight decline in the perivitelline fluid, as the yolk expanded into the perivitelline space. The perivitelline space, together with the chorion was estimated in the 50-day-old eggs by tritiated water exchange (see below). After 50 days the water

content of the egg had increased by more than a third. No consistent differences in the properties of fertilized and unfertilized eggs were detected in eggs up to a few days old, but the following data refer to fertilized eggs unless otherwise stated.

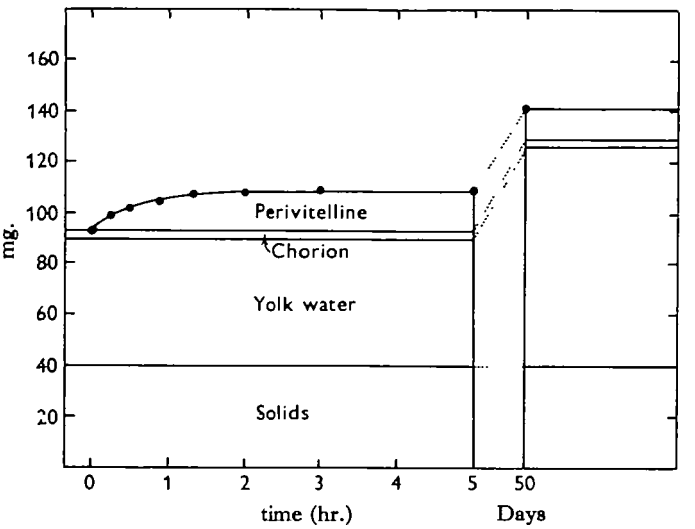

Fig. 1. Changes in the weight and the distribution of water in an unfertilized egg of *Salmo salar* when stripped into Lune river water. First 5 hr. 3·4° C, later period 10° C.

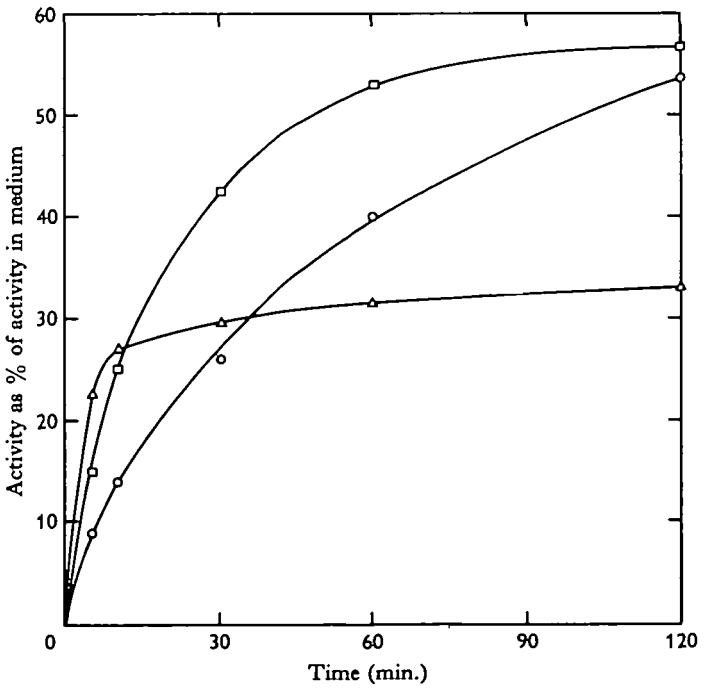

Fig. 2. Tritiated water exchange in eggs of *Salmo salar*. □, Freshly stripped fertilized eggs in Lune river water. ○, Freshly stripped egg in salmon saline. △, Water-hardened eggs in Lune river water. 3·4° C.

Exchange of tritiated water in water-hardened eggs

When water-hardened eggs were placed in tritiated water the specific activity of the whole egg increased as shown in Fig. 2. The initial rapid increase was probably due to the equilibration of water in the chorion and the perivitelline fluid with that in the external medium. The very slow rise in activity which followed implies that some further exchange of water took place between the yolk and the external medium. The volume of the perivitelline fluid, plus chorion, calculated from the rapidly exchanging fraction of water (27%) agrees well with that determined directly from the increased weight of the egg, etc. (25%) (Fig. 1). In water-hardened eggs a few hours old, the rate constant for the exchange of water between the yolk and the external medium calculated from the slow exchange lies between 0·02 and 0·03/hr. In one experiment with 50-day-old unfertilized eggs it was 0·023/hr. but in 40-day-old eyed eggs it was as high as 0·05/hr. Once water-hardening is complete the permeability is evidently constant for a long period.

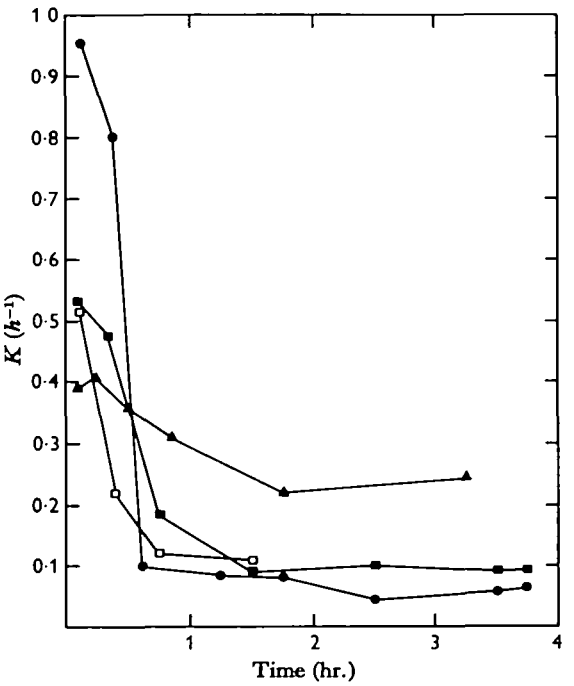

Fig. 3. Exchange constant of water across the vitelline membrane of freshly stripped fertilized eggs of *Salmo salar* placed in various media. ●, Isosmotic glucose. ▲, Isosmotic saline. ■, River water. □, Distilled water. 3·4° C.

Exchange of tritiated water in fresh eggs and the process of water-hardening

The increasing specific activity of water in freshly stripped eggs after transfer to river water containing ^3H_2O is also shown in Fig. 2. The higher activity was due to the initial rapid influx of tritiated water into the yolk whilst the eggs were still highly permeable. It is clear from Fig. 3 that the initial permeability of the vitelline membrane to water is very high. For reasons previously explained the initial value is most subject

to error. However, even if an error is made in the initial assumptions this will have little effect on successive values of K which are based on the changes in weight and on the specific activity of the whole egg. The permeability begins to decline almost at once, and continues to decline for 7 or 8 hours. After that it remains virtually constant for 50 days in unfertilized eggs.

Permeability of eggs in isotonic saline

Eggs transferred after stripping to isotonic saline maintain a constant weight. However, during the first 3 hr. there was some detectable decline in the permeability of the vitelline membrane to water, although the exchange constant never declined below about 0·2/hr. (Fig. 3). This decline in permeability appears to be real, as the specific activity of water in the saline eggs reaches 20% of that of the external medium within twenty minutes (Fig. 2). As there is no increase in the weight of the egg, and therefore no formation of the perivitelline fluid, it is doubtful whether surface contamination, together with activity in the chorion and any pre-existing perivitelline fluid, could account for this high activity. If the initial value of the exchange constant of the yolk water was only 0·2/hr., the rapidly exchanging water fraction (chorion plus any perivitelline fluid plus contamination) in the saline egg would have to amount to 20% of the total egg water, or 10 mg. in order to account for the early values observed in Fig. 2, not the 3 mg. assumed in the calculation of Fig. 3. It seems more likely that the exchange constant of the freshly stripped egg is about 0·4/hr., and it soon declines to about 0·2/hr. in saline.

Permeability of eggs in isotonic glucose

As Bogucki observed in 1930, the behaviour of eggs in isotonic non-electrolytes at first sight is anomalous. In isotonic glucose the eggs gained weight more rapidly than in river water. After 45 min. the process of swelling was complete, whereas it continued for about 2 hr. in river water. Eggs initially weighing 93·6 mg. attained a maximum weight of 113 mg. in glucose and only 108 mg. in river water. The increase in the specific activity of the whole egg in glucose solutions containing tritiated water was similar to that in tritiated river water but detailed calculations show that the decline in the permeability (Fig. 3) was even more rapid than in river water. The initial value of the computed exchange constant in glucose is very high. Again this might be an artifact due to an inadequate allowance for the chorion, etc., but calculations show that the chorion and other rapidly exchanging water fractions again would have to amount to no less than 20% of the total water content of the egg during the first few minutes to bring the permeability constant into line with the permeability of saline eggs a few hours old, *ca.* 0·2/hr.

The influence of sodium and calcium ions on the rate of water-hardening

Gray (1932) found that the presence of calcium ions seemed to help to maintain the low permeability of the vitelline membrane in the presence of heptyl alcohol. To test the influence of sodium and calcium ions on the process of water-hardening, fresh eggs were transferred into the solutions shown in Fig. 4. From the weight changes and the specific activities of the eggs the permeability constants of the vitelline membrane were calculated as before. The results are shown in Fig. 5. Although the rate of

increase in weight was similar in many of the dilute solutions, analyses showed that the behaviour of the eggs differed markedly in the different media. The formation of the perivitelline fluid was most rapid and most extensive in distilled water (Fig. 4*a*) but water-hardening, as shown by the decline in K, was also the most rapid in this medium (Fig. 5). The activity in the yolk therefore remained low. In the presence of 145 mM-Na/l. the formation of perivitelline fluid was completely inhibited and water-

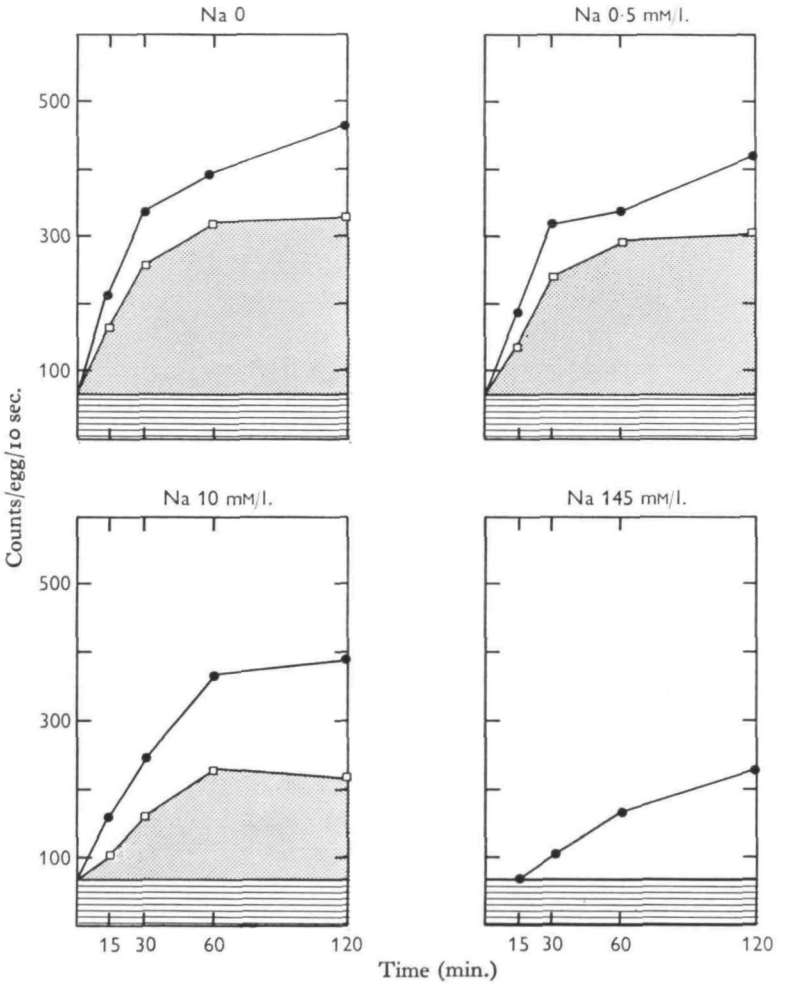

Fig. 4(*a*). No Ca.

Fig. 4. Distribution of activity in the water compartments in eggs of *Salmo salar* when placed in solutions containing tritiated water and various concentrations of sodium and chloride ions. Activity in chorion ▤. Activity in perivitelline fluid ▦. Activity in yolk water □. 3·4° C.

hardening was delayed. The activity in the yolk reached a higher level after 2 hr. and was continuing to increase. A low concentration of calcium, 0·5 mM/l., reduced both the rate of formation and the final volume of the perivitelline fluid (Fig. 4*b*). A medium with 10 mM/l. of calcium delayed the formation of the perivitelline fluid even further, but 10 mM/l. of sodium alone had less effect (Fig. 4*a*).

0·5 mM calcium/l. significantly delayed the reduction in the permeability of the vitelline membrane (Fig. 5) and the activity in the yolk, therefore, reached a high level after 2 hr. (Fig. 4*b*). 10 mM/l. of calcium was no more effective, indeed the decline in *K* was more rapid in 10 mm. Ca alone than in 0·5 mM-Ca (Fig. 5). 0·5 mM-Na/l. has no significant effect in reducing the rate of water-hardening (Fig. 5) but 10 mM-Na/l.

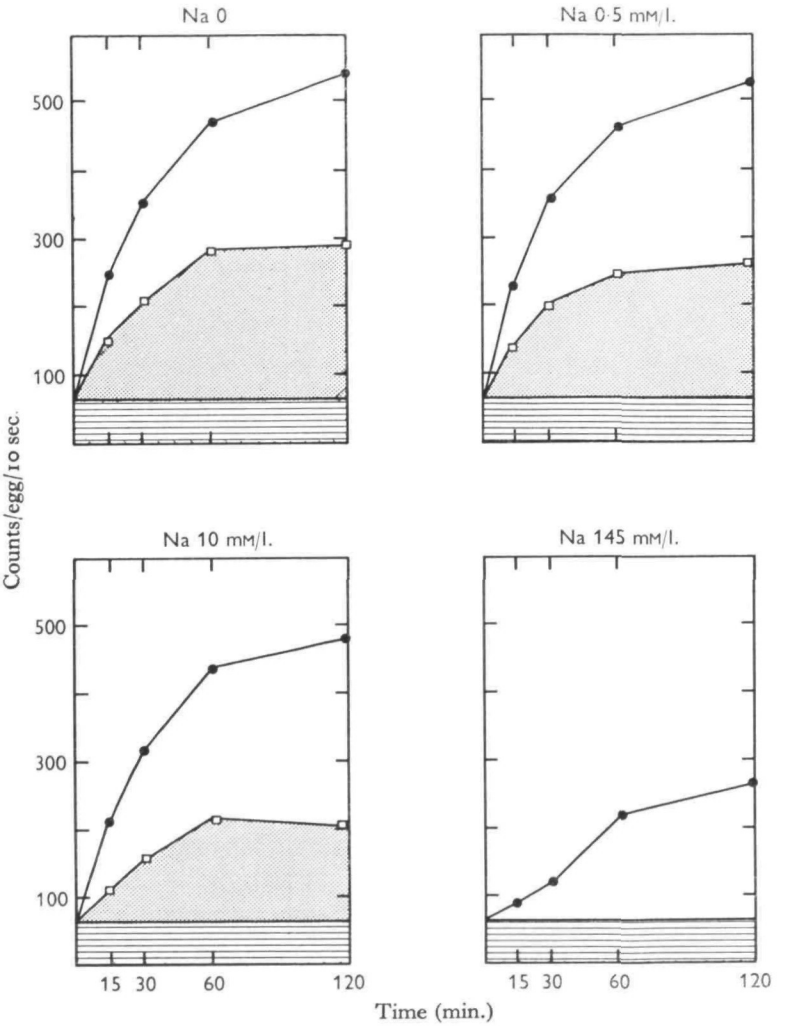

Fig. 4(*b*). 0·5 mM Ca/l. For legend see Fig. 4(*a*).

alone does reduce it and the permeability of the vitelline membrane after 2 hr. approximated to that in isotonic saline after 2 hr. (Fig. 5). Although 0·5 mM-Na/l. has little or no effect on the permeability of the vitelline membrane it does reduce the rate of formation and final volume of the perivitelline fluid (Fig. 4*a*, *b*). An even lower concentration of calcium, equivalent to that in river water, would be effective in both delaying the process of water-hardening and reducing the rate of formation and the final volume of the perivitelline fluid, while a similar concentration of sodium ions will

only reduce the volume of perivitelline fluid. Thus calcium ions appear to be more effective in both respects than sodium ions. However, the calcium solutions of equivalent molarity always have a higher total osmotic concentration than equivalent sodium solutions because calcium is a divalent ion. It is impossible to decide on these data whether the difference between sodium and calcium is due to an ionic or osmotic effect.

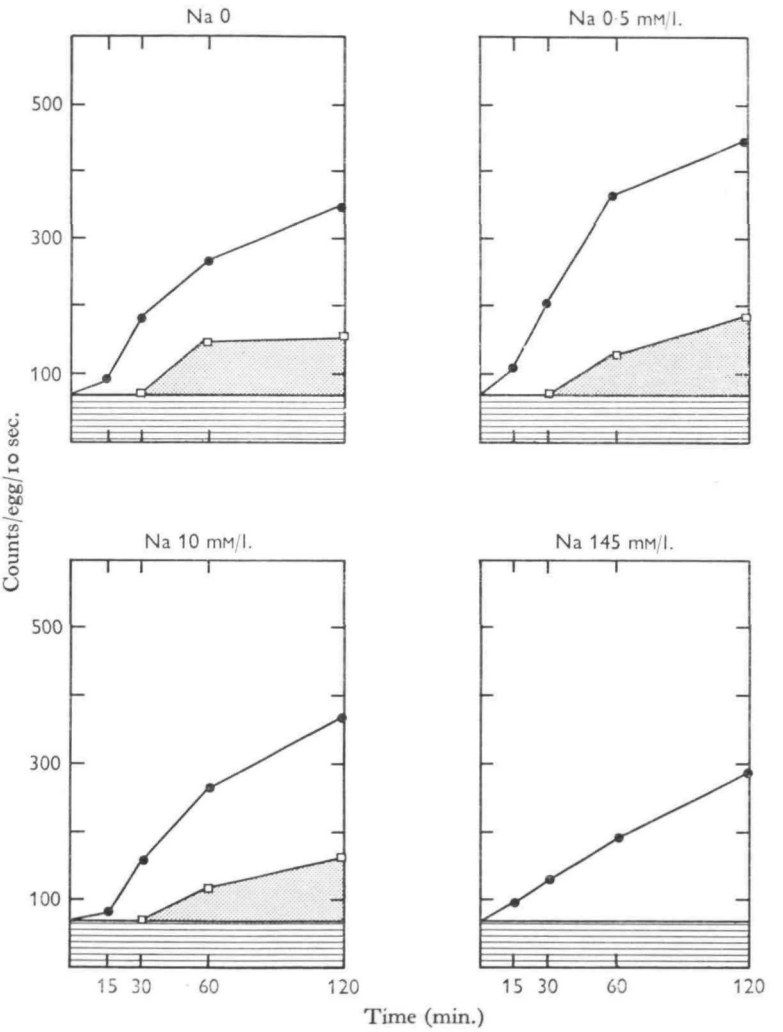

Fig. 4(*c*). Ca 10 mM/l. For legend see Fig. 4(*a*).

Vitelline membrane and cytoplasm as barriers to diffusion

It is often assumed that the barrier to the salt and water movements in all cells lies in the cell membrane, but Ling, Ochsenfeld & Karreman (1967) have shown recently that the barrier to the diffusion of tritiated water in the frog ovarian egg lies not in the egg membrane but in the cytoplasm. Hence the process of water-hardening may not be a decrease in the permeability of the vitelline membrane but either a change in the properties of the cytoplasm or the appearance for the first time of a relatively imperme-

able barrier at the vitelline surface. Ling *et al.* have been able to distinguish between the case where the rate of diffusion is limited by the properties of the surface membrane and where it is limited by the bulk properties of the cytoplasm, by plotting the specific activity of the cytoplasm water against the square root of time. When diffusion is surface-limited the plot shows an inflexion; when it is bulk-phase-limited there is no inflexion. That is to say, diffusion into the cytoplasm is initially more rapid when it is

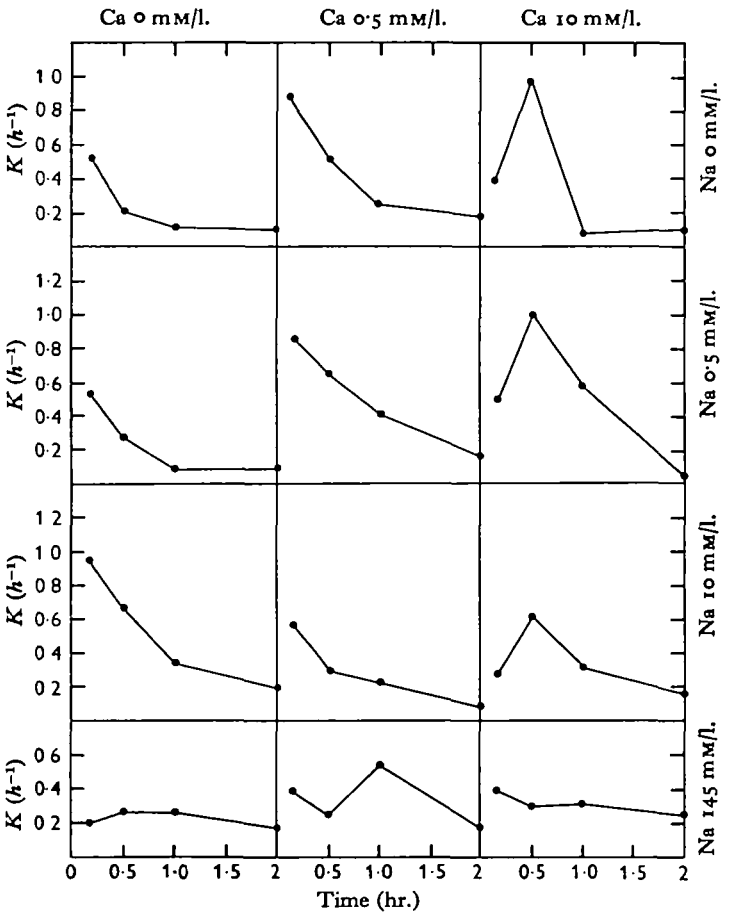

Fig. 5. Changes in rate constant of exchange of yolk water in freshly stripped salmon eggs placed in various concentrations of sodium and chloride ions calculated from the data in Fig. 4.

Note: In the solution containing zero calcium and 0·5 mM-Na/l, a fortuitous accumulation of small errors caused the activity in the yolk apparently to decrease between 30 min. and 1 h. (Fig. 4a). *K* has therefore been calculated in this case from the increase in activity between 30 min. and 2 hr. and has been assumed constant between these times.

bulk-phase-limited than when it is surface-limited. It would remain difficult to distinguish between these two possibilities in the water-hardened state because it is always necessary to allow for the relatively large volume of water present in the perivitelline fluid and in the chorion. Any small error in this allowance might well introduce an inflection into the early part of the curve.

In an attempt to distinguish between these two possibilities the specific activities

of the egg water at different times were measured in eggs in saline (Fig. 6*a*). The first section of the graph approximates to a straight line, and the slight rise at the first point may be due to surface contamination. However, the vitelline membrane lies beneath the chorion some distance inside the surface of the egg. Diffusion into the egg in the first 2 or 3 min., mainly into the chorion, will therefore be bulk-phase-limited. The chorion accounts for 6% of the whole egg water. When allowances are made for this chorionic water the influx curve of the tritiated water into the yolk shows a distinct inflexion, implying that the vitelline membrane is a barrier to diffusion (Fig. 6*b*), even in the freshly stripped egg.

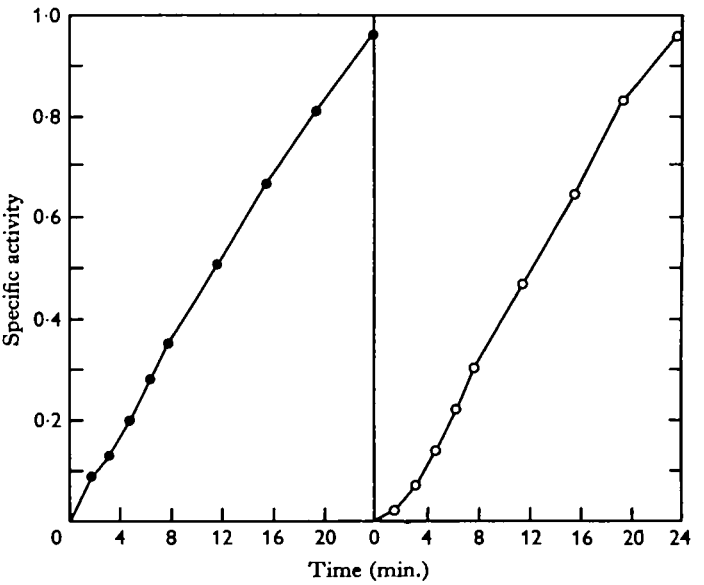

Fig. 6. Increase of specific activity of water with time in freshly stripped eggs in saline. (*a*) Whole eggs ●, (*b*) Yolk water ○. 3·4° C.

DISCUSSION

Permeability of the vitelline membrane

Although Zotin (1965) suggests that the low rate of exchange of water in salmon eggs may be due to a peculiarity of the physical state of the water the present results are most easily interpreted in terms of a change in the permeability of the vitelline membrane as originally suggested by Gray. It is difficult to assess the permeability of the egg when it is in the body cavity. The saline solution was intended to replace the coelomic fluid of the fish. When the egg is transferred directly into the saline solution, the exchange constant of the egg is initially about 0·4/hr., but even in the saline after 2 hr. it drops to about 0·2/hr. This decline indicates that the saline is not exactly equivalent to the coelomic fluids of the fish. When transferred to de-ionized water or into salt-free glucose or dilute solutions of sodium or calcium ions the exchange constant (K) rises as high as 1·0/hr., but then declines rapidly to about 0·1 or 0·2 after 2 hr. and eventually to about 0·025 after 10 hr. While it is possible that the exchange constant of eggs in the body cavity of the fish is normally as high as 0·6–1·0/hr., the

results of the experiment with calcium solutions suggest that this is not the case. When eggs are transferred into solutions containing little or no sodium but 10 mM of calcium (Fig. 5), the value of K during the first 15 min. is about 0·4–0·5/hr., but then rises to a high level of 1·0/hr. before declining again. This suggests that the resting value of K is about 0·4/hr., and that transfer into dilute solutions causes an initial increase of the permeability before it finally declines. Calcium ions appear to delay the onset of the stage of high permeability for a short time while high sodium concentrations delay the onset indefinitely. If the activity within the chorion, external to the vitelline membrane, is over-estimated this will produce a spuriously low value for K during the first period of time. While the low value of K during the first period of time in calcium solutions may be an artifact due to some error in estimating the activity external to the vitelline membrane, it is noteworthy that the formation of perivitelline fluid is also delayed by the presence of 10 mM of calcium (Fig. 4*c*). The formation of the perivitelline fluid is assessed unambiguously from the changes in weight of the egg (Fig. 1). The decline in permeability is most rapid in distilled water, or in salt-free solutions of glucose, or in solutions containing little sodium and no calcium. The permeability of the vitelline membrane is apparently greatest in solutions containing 0·5–10 mM-Ca/l. and 0–10 mM of Na/l. Similar high values of K may be reached in calcium-free solutions during the first few minutes but the permeability may decline so rapidly that the mean permeability during the 15 min. is lower. In solutions containing 10 mM-Ca/l. highest permeability occurs between 15 and 30 min. after transfer to the solution. For reasons discussed above it is unlikely that these high values are a product of spurious assumptions. There is some evidence of a decline in permeability in solutions containing 145 mM sodium but low calcium. The most constant permeability is maintained in the solutions containing high concentrations of both sodium and calcium, but the exchange constant is only about 0·3/hr.

Although the permeability is relatively high before laying and during the process of water-hardening, in absolute terms it is always remarkably low, and falls to almost negligible values once the egg is water-hardened. From the surface area of the vitelline membrane, and from K, it can be calculated that the permeability of the egg before shedding is probably about 0·06 to μ^3/μ^2/sec., and even at its highest it does not rise above 0·2 μ^3/μ^2/sec.* In water-hardened eggs the permeability is $< 0{\cdot}004$ μ^3/μ^2/sec. In comparison the permeability of the surface of *Amoeba* is 0·35/μ^3/μ^2/sec. (Presscott & Zeuthen, 1953). The surface of the alder fly larva, *Sialis lutaria*, has a permeability of between 0·04 and 0·05 μ^3/μ^2/sec. (Shaw, 1955). In contrast, ovarian eggs of the frog have a permeability of as high as 89 μ^3/μ^2/sec., but this declines to 1·2 μ^3/μ^2/sec. before laying (Hevesy, Hofer & Krogh, 1935). In *Valonia ventricosa* permeability is 2·4 μ^3/μ^2/sec. (Gutknecht, 1967). The very low permeability of the salmon egg is no doubt related to its prolonged development in fresh water. Even this low permeability to water results in a slow but continuous swelling of the yolk during development. In the unfertilized egg this is accompanied both by an increase in total volume of the egg, but also by a reduction in the volume of perivitelline fluid. In the course of 50 days the volume of the water in the yolk increases by about 36 mg. or 0·072 mg. per day. Water-hardened eggs during the period contain on average 70 mg. of water which exchanges with a rate constant of 0·025/hr. The total water exchange is therefore

* See Appendix.

42 mg./day. The estimated osmotic influx, calculated from the total water flux and the osmotic concentration of the yolk, is 0·019 mg. water/day. The real osmotic influx is 0·72 mg./day or nearly four times greater than that calculated from the diffusion flux. This discrepancy probably indicates the presence of pores through which water flows into the yolk (Koefoed-Johnsen & Ussing, 1953), although some part of the discrepancy may be due to the presence of unstirred layers at the surface (Dainty & House, 1966). In the fertilized egg the permeability to water increases again in the eyed stage, about 40 days, but is still very low in absolute terms: K is only 0·05/hr. This is twice that for water-hardened unfertilized eggs of the same age but still very low by comparison with most freshwater organisms. This increase may be due at least in part to the increased surface area of the embryo rather than to increased permeability *per se*.

Formation of the perivitelline fluid

In the water-hardened egg the perivitelline fluid forms 22% of the weight. All the water in the perivitelline fluid together with the capsule water exchanges within a few minutes with the external medium (Fig. 2). Formation of the perivitelline fluid is almost complete within 1 hr. of shedding into distilled water or into solutions containing little sodium; but in solutions of 145 mM-Na/l., approximately isotonic with the body fluid, the formation of perivitelline fluid is completely suppressed, and the eggs then maintain a constant weight. However, when the eggs are shed into salt-free isosmotic glucose solutions, the formation of the perivitelline fluid takes place more rapidly than in river water. Bogucki observed many years ago that perivitelline fluid appeared when eggs were shed into isosmotic solutions of urea or glucose. The glucose solution is equivalent to distilled water for both perivitelline fluid formation and permeability reduction. Even 0·5 mM/l. of sodium or calcium slightly reduces the rate of formation of the perivitelline fluid, while 10 mM calcium/l. markedly reduces it. Just as calcium delays the onset of the phase of high permeability, calcium also delays the onset of the formation of perivitelline fluid (Fig. 4*c*). The parallel between formation of perivitelline fluid and the state of high permeability cannot be an artifact of the assumptions in the calculations. If the volume of perivitelline fluid were underestimated at any time, this would produce a spuriously high value for the activity within the yolk water and hence of the permeability and vice versa.

The process of perivitelline fluid formation is obscure but the fluid exerts an appreciable osmotic pressure and contains some substance which concentrates sodium ions (Rudy & Potts, 1968). Bogucki (1930) suggested that the yolk liberated a colloidal substance into the perivitelline space at the time of shedding which then took up water to form the perivitelline fluid. In *Fundulus heteroclitus* the formation of the perivitelline fluid coincides with the disappearance of a layer of small clear vesicles from immediately below the vitelline membrane (Kao & Chambers, 1954). It is likely that the phase of high permeability in *Salmo salar* is related to the release of the perivitelline colloid which must temporarily disrupt the integrity of the vitelline membrane. Electron microscopy might elucidate this point.

From the ratio of the osmotic to the diffusional influx (Solomon, 1959) the diameter of the pores may be estimated at 6·3 Å, similar to that in the erythrocyte. However, the osmotic permeability of the erythrocyte to water is 125 μ^3/μ^1/sec. (Dick 1959), implying a much higher density of pores. It is not possible to calculate

the number of pores necessary to account for the observed permeability without making some assumption about their length. Solomon estimated that the pores in the erythrocyte averaged 300 Å long and occupied 6×10^{-4} of the surface of the cell. On the same assumption a crude calculation suggests that the water-hardened salmon egg would possess 10^8 pores occupying 3×10^{-7} of the egg surface. The reduction in permeability which accompanies water-hardening may be related either to a reduction in pore numbers or in pore diameters. These two possibilities could be distinguished if accurate measurements could be made of the osmotic permeability of the vitelline membrane during the short-lived period of high permeability but this would be very difficult in practice.

Calcium or other alkaline earth ions are often necessary for the maintenance of a state of low permeability in biological membranes (e.g. frog bladder, Bentley, 1959). The delaying effect of calcium ions on the permeability changes in salmon eggs is unusual. There is evidence that calcium ions play an important role in the regulation of ion permeability in amphibian oocytes (Morrill, Rosenthal & Watson, 1966) as well as in nerve muscle tissue, but the vitelline membrane of salmon eggs is effectively impermeable to sodium ions at all times during the process of water-hardening (Rudy & Potts, 1968).

SUMMARY

1. The rate of exchange of tritiated water across the vitelline membrane of the eggs of the Atlantic salmon has been examined under various conditions.

2. In eggs shed into isosmotic saline the rate constant of exchange is about 0·3–0·4/hr.

3. When eggs are shed into distilled water or isosmotic glucose rate constants as high as 0·8–1·0/hr. occur for short periods but the rate of exchange declines rapidly.

4. Low concentrations of sodium and more especially of calcium ions delay both the phase of rapid exchange and the phase of declining permeability.

5. The rate of formation of perivitelline fluid is greatest during the period of high permeability.

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APPENDIX

The absolute permeability of the vitelline membrane may be estimated as follows:

It is assumed that the egg, without the chorion, weighs 90 mg., has a density of 1·0 and contains 50 mg. of water.

The radius (r) of the vitelline membrane is then 2·78 mm., as $\frac{4}{3}\pi r^3 = 90$.

The area of the vitelline membrane ($4\pi r^2$) is then 97·6 mm.^{2}.

If the rate constant of water exchange in freshly stripped eggs is $0{\cdot}4h^{-1}$ then the water flux/sq. mm./hr. $= \dfrac{0{\cdot}4 \times 50}{97{\cdot}6}$.

Hence water flux in

$$\mu^3/\mu^2/\text{sec.} = \frac{0{\cdot}4 \times 50}{97{\cdot}6} \times \frac{1000}{3600} = 0{\cdot}057.$$

Similarly, if the rate constant for water hardened eggs $= 0{\cdot}025h^{-1}$ the permeability $= 0{\cdot}0035\ \mu^3/\mu^2/\text{sec.}$ and if the rate constant rises to $1{\cdot}0h^{-1}$ during the period of high permeability then the absolute permeability on these assumptions $= 0{\cdot}14\ \mu^3/\mu^2/\text{sec.}$