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WATER EXCHANGE IN THE PIKE EGG

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INTRODUCTION

The eggs of various teleost species have frequently been used to study the factors governing osmoregulation in a hypotonic environment. As a result of some of these investigations the general impression arose that these eggs became impermeable to water after shedding, remaining so until advanced developmental stages. Recent reports of water exchange in *Salmo salar* eggs, however, have shown that this view should be modified (Loeffler, 1968; Potts & Rudy, 1969; Loeffler & Løvtrup, 1970). Further evidence in support of this need for modification has been obtained in an additional teleost species, *Esox lucius*.

When pike eggs are shed in fresh water they become activated, as in salmonid species; the perivitelline compartment forms – partly by water uptake from the environment – and the chorion is transformed into a rigid structure. Thereafter volume and water content are confined within narrow limits, but such observations cannot decide for or against water movement across the cell surface. The problem of demonstrating water permeation, even under conditions of constant volume, can be readily solved using isotopic water; by following water exchange with the automatic diver-balance both permeability and water content can be determined.

In a series of preliminary experiments it became evident that the extent of water exchange in pike eggs represented considerably more than the content of the perivitelline compartment (Loeffler & Løvtrup, 1969), indicating that at least a part of the water phase of the egg proper was also susceptible to exchange with the surrounding medium. Comparisons of the amount of exchangeable water from these 'pilot' experiments with determinations of water content based upon wet–dry weight measurements were consistent with the notion that essentially the entire water phase of the egg could be replaced by the external medium.

These observations have provided the impetus for the present report: to determine the diffusion and exchange coefficients of water during the developmental interval from egg shedding to advanced myomere embryos. The results are consistent with our preliminary findings regarding water turnover, and support some older statements concerning the importance of the activation process in the osmoregulation of teleost eggs. Finally, a number of similar isotope-exchange studies are considered with the aim of comparing the exchange coefficients of eggs and embryos during early development.

MATERIALS AND METHODS

General considerations; handling and preparation of eggs and embryos. Male and female *Esox lucius* were caught in the vicinity of Umeå during the breeding season.

Eggs were 'stripped', divided into two groups, one of which was fertilized, and stored in 7·5 % or 100 % Ringer solution at 10 °C. As in *Salmo*, activated (= hardened) eggs can be obtained in the absence of fertilization by immersing freshly shed eggs in 7·5 % Ringer. When freshly shed eggs are placed in media of relatively high salt content, such as 100 % Ringer, the chorion does not become rigid and the formation of the perivitelline compartment can be partially inhibited. The latter procedure has been employed to obtain 'unhardened' eggs.

Prior to experimentation the eggs were freed of adhering debris and permitted to attain the test temperature of 9·0 °C. Before and at the end of each determination several measurements were made with an ocular screw micrometer of the diameters described by the chorion and the egg surface proper at two planar views; using these average values, the volumes of the whole egg, the egg proper and the perivitelline compartment can be calculated. The percentage water content of eggs and embryos was estimated at each developmental stage investigated by conventional wet–dry weighings of groups of 20 specimens.

Measurements of water exchange. Water exchange was followed by the isotope method introduced by Pigon & Zeuthen (1951) and Løvtrup & Pigon (1951) using a modified automatic electromagnetic diver-balance developed by Larsson & Løvtrup (1966). In the present work the reduced weight (RW) of known standards and the RW of the objects undergoing exchange were corrected for deviations in the floating level of the diver; when such corrections are applied to the calibration curves very close estimates of the reduced weight can be realized.

All measurements were conducted in either 7·5 % or 100 % Ringer solution containing 20 % heavy water. Individual eggs were placed on the balance and RW changes were determined at convenient time intervals until they began to level off, an indication that egg water had been replaced by the isotopic mixture. The corrected changes in RW were plotted as a function of time in order to calculate the first-order constant, k, giving the best fit to the experimental points (Guggenheim, 1926). Using k and selecting two points on the theoretical curve, RW_∞, ΔRW, 'RW_0' and RW_0 were determined; the symbols will be explained in the text. From the overall change in reduced weight (ΔRW), the amount of exchanged water can be obtained: $V_w = \Delta RW / 0{\cdot}20\,(\phi_{D_2O} - \phi_{H_2O})$; where 0·20 is the percentage of heavy water in the test medium; ϕ_{D_2O} and ϕ_{H_2O} are the densities of heavy and ordinary water. These values of V_w have been used as estimates of the water content of the egg, and compared with the wet–dry weight determinations.

Calculations of the diffusion and exchange coefficients. The rate of water exchange between a cell and its surroundings can be expressed in terms of the rate at which water passes through the cell membrane and the rate of water diffusion inside and outside the cell (Hansson Mild, 1971 *a*, *b*). In the present investigations diffusion in the outside medium has been neglected in the mathematical considerations, and therefore our estimates of water diffusion in cytoplasm (the diffusion coefficient, D) may be somewhat lower than the actual values. It will be shown, however, that the use of these determined D values does not appreciably alter the value of the exchange coefficient, E.

In order to estimate E in unfertilized and normally developing eggs the diffusion coefficient for water in cytoplasm must be known. It has been assumed that this requirement can be met by measuring exchange in a cell deprived of its surface

resistance to water flow, and then using the determined value of D to estimate E. Both natural and artificial means have been employed to obtain such 'cells'. Although it is difficult to assay the effect of such treatments (Haglund & Loeffler, 1969; Løvtrup, Hansson Mild & Berglund, 1970), formalin has been used in the present experiments as described previously (Haglund & Loeffler, 1969). Fertilized, hardened eggs that had completed the first two or three cleavages were used to obtain D, and these values were used as estimates of water diffusion through cytoplasm at all stages examined.

The values of D and E have been calculated according to Løvtrup (1963). In the absence of a surface barrier to water movement, the formula for calculating D is: $2{\cdot}303 \times k \times R^2/\beta_1^2 \times 60$. When a diffusion barrier is present at the surface the first-order constant, k, is equal to $\beta_1^2 \times D \times 60/R^2 \times 2{\cdot}303$ (60 is introduced to convert minutes to seconds and 2·303 to convert from natural to common logarithms; the other symbols are explained in the text). The average value of the radius (R) corresponding to the chorion was used to determine D; the radius of the egg proper was used to calculate E.

RESULTS

Determinations of the diffusion coefficient. Results obtained with a single formalin-treated egg are shown in Fig. 1 A. The changes in RW followed a time course similar to that observed in salmon eggs treated with alcohol (Loeffler & Løvtrup, 1970). Rapid exchange during the first 2–3 min (dashed lines) is not accounted for by the theoretical curve (continuous line) as the latter represents the first approximation of an infinite series describing diffusion in a sphere without a surface barrier (Crank, 1956). In the absence of a surface restriction ($L \to \infty$; $E \to \infty$) a difference exists between the two equations (i.e. the first approximation and the infinite series) that gives a value of $RW_\infty - 'RW_0'$ that is too low by a factor of $\pi^2/6$ (Løvtrup, 1963). Thus in the present example, the overall change in reduced weight ($\Delta RW = RW_\infty - RW_0$) would be $(RW_\infty - 'RW_0')\pi^2/6 = 170\ \mu g$. The actually observed 'zero-point', RW_1, determined by the diver-balance, was slightly above the theoretical value RW_0. It should be emphasized, however, that this initial determination by the balance is not the 'true zero-point', but merely serves as an approximation of the null position.

The above observations support the interpretation that water exchange is proceeding in the absence of a surface restriction (= surface barrier) to water flow. The diffusion coefficient was found to be $5{\cdot}8 \times 10^{-6}\ cm^2\ sec^{-1}$, half-time for the exchange being about 4 min. The amount of water exchanged (V_w) was estimated to be 8·5 μl, a water content of approximately 77 %, as compared with 79 % determined by wet–dry weight measurements of formalin-treated eggs of the same stage. The close agreement between these two estimates supports the view that a large part of the water content of the whole egg – perivitelline compartment and egg proper – has been exchanged; the same indications were observed in all other experiments here reported.

Additional results concerned with determinations of chemically treated eggs are contained in Table 1. Values of D in dilute Ringer were higher than that reported for *Salmo salar* ($\sim 4 \times 10^{-6}\ cm^2\ sec^{-1}$, at 5·5 °C); the variation could be accounted for by differences in temperature and method of treatment (Loeffler & Løvtrup, 1970). The mean values of D in 7·5 % and 100 % Ringer were 6·2 and $5{\cdot}0 \times 10^{-6}\ cm^2\ sec^{-1}$, respectively. It is suggested – on the basis of the isotope-exchange data – that this

difference is the reflexion of the slightly higher percentage water content of the egg tested in 7·5 % Ringer. Estimates of the water volume per specimen by isotope exchange in dilute Ringer ranged between 77 % and 83 %, as opposed to 70 % and 76 % in full-strength salt solutions. As the water content of the latter is in closer agreement with the water content of normally developing eggs, it could be argued that the value of D obtained in 100 % Ringer is a more accurate estimate of water diffusion through cytoplasm. The solution of this problem is quite complicated, and since it turns out that small differences in D do not affect the value of E in the present calculations, the diffusion coefficients obtained at the two tonicities have been used in the corresponding determinations of the exchange coefficients that follow.

Table 1. *Data pertaining to the formalin-treated eggs of* Esox lucius, *at* 9·0 °C

Egg specimen and tonicity of Ringer solution		Calculations from isotope-exchange determinations related to: The diffusion coefficient: R (mm)	k ($\times 10^3$ min^{-1})	D ($\times 10^6$ cm^2 sec^{-1})	The water content: ΔRW (μg)	V_w (μl)	E_v* (mm^3)	% water ($V_w/E_v \times 100$ %)
7·5 %	1	1·38	78	5·8	170	8·5	11·0	77
	2	1·36	98	7·1	173	8·7	10·5	83
	3	1·35	79	5·6	163	8·1	10·3	79
		(Mean ± S.D.: 6·2 ± 0·8.)						
100 %	4	1·39	70	5·3	158	7·9	11·3	70
	5	1·35	65	4·6	155	7·8	10·3	76
		(Mean ± S.D.: 5·0 ± 0·5.)						

* E_v represents the volume of the whole egg; the other symbols have been explained in the text.

Determinations of the exchange coefficient. The characteristic feature of the following experiments is the relatively lengthy time interval required before the changes in RW began to level off. As distinct from the previous determinations, water exchange proceeded in two nearly separate steps: (1) an initial rapid turnover lasting several minutes, and (2) a slower exchange that continued for 2 or more hours. The experimental points of the latter fit a curve indicative of water exchange in the presence of a surface barrier. Since the exchange is slow ($L < 1$), the first approximation and the infinite series describing exchange are nearly coincident, and therefore 'RW_0' $\cong RW_0$ (Løvtrup, 1963). The first obtainable point by the diver-balance, RW_1, represents an approximation of the 'zero' position indicating the beginning of water exchange in the perivitelline compartment.

An experiment with an unhardened egg is shown in Fig. 1 B. The initial rapid exchange in reduced weight ('RW_0' $- RW_1$) indicates that a perivitelline compartment has formed – an observation confirmed by microscopy and by the easily deformable nature of the chorion. Thus, the gross morphological changes proceeding from egg-activation, perivitelline compartment formation and chorion hardening, have been 'dissociated'. The significance for osmoregulation will be discussed later, but it can be mentioned here that a continued exposure to full-strength Ringer solution for 6 h, or longer, resulted in 'swelling' of the chorion and the egg proper.

After this primary exchange the rate decreased, and RW changes gave evidence of

reaching a plateau after 2 h. The calculated curve intersects the ordinate axis at 'RW_0' = 45 μg, and the overall change in reduced weight corresponds to 109 μg. The part of the water exchange not accounted for by the curve (2·3 μl) is about 40 % of the exchangeable water of the whole egg (5·5 μl; Table 2). If the initial rapid change in RW (45 μg) represents water turnover in the partially formed perivitelline compartment, the remaining change in reduced weight ($RW_\infty - $'$RW_0$' = 64 μg) is the exchange-turnover of the egg proper. The water volume by isotope exchange (V_w) was 64 %; wet–dry weight determinations yielded 70 %. These observations are instructive when compared with similar measurements of water volume in hardened and normally developing eggs, for they indicate not only that activation results in chorion transformation and water uptake in the perivitelline compartment, but also that water is taken up by the egg proper.

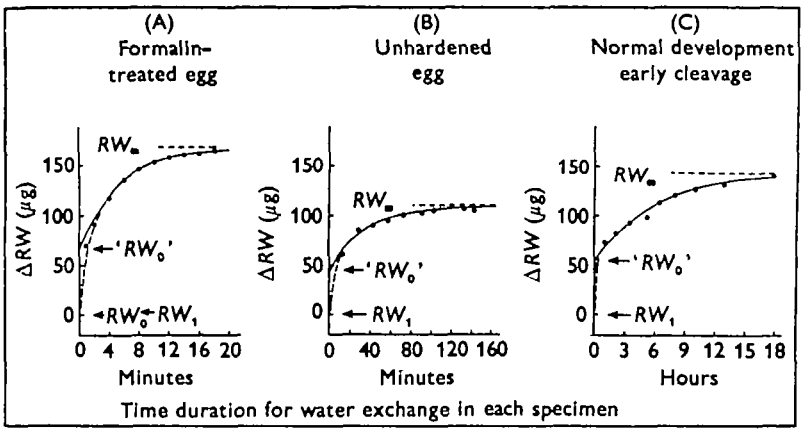

Fig. 1. Isotopic water exchange as determined by the automatic diver-balance in three different experiments, at 9·0 °C: (A) formalin-treated egg tested in 7·5 % Ringer; (B) unhardened egg in 100 % Ringer; (C) a normally developing egg in early cleavage, tested in 7·5 % Ringer solution. The curves shown by continuous lines are the first approximations of those infinite series representing water exchange in a sphere without (A) and with (B, C) a surface barrier. RW_∞ and 'RW_0' are the calculated values at $t = \infty$ and $t = 0$; 'RW_0'$-RW_0$ is a mathematical artifact; RW_1 is the recorded 'zero-point' obtained by the balance. The value 'RW_0'$-RW_1$ in B and C is an approximate measure of the exchange of the water phase of the perivitelline compartment.

Using the mean value of D obtained in 100 % Ringer solution the exchange coefficient of this unhardened egg was calculated to be $1{\cdot}7 \times 10^{-5}$ cm sec^{-1}, half-time for the exchange being 30 min. Of interest in this regard is the value of L, which can be used as an index of the 'tightness' – the resistance to water flow – of the membrane. When $L < 1$, as it is in the present example, the equation describing water turnover is closely approximated by the first term of an infinite series with the exponent equal to: $-D\beta_1^2 t/R^2$; D and R have been defined previously; t is the time in seconds; β_1^2 is defined by the equation $\beta_1 = (1-L)\tan\beta_1$ (Løvtrup, 1963). The dimensionless parameter L is given by $L = RE/D$. To show that the value of the diffusion coefficient does not appreciably alter the value of E reported in these investigations, the following calculations have been made. E values have been recalculated for the present example

Table 2. *Data pertaining to the unfertilized and normally developing eggs of* Esox lucius, *at* 9·0 °C

Developmental stage and tonicity of Ringer solution	Calculations from the isotope-exchange determinations related to: The exchange coefficient: R (mm)	k ($\times 10^3$ min^{-1})	β_1	L	E ($\times 10^6$ cm sec^{-1})	The water content: ΔRW (μg)	V_w (μl)	E_v (mm^3)	% water ($V_w/E_v \times 100$ %)
Unfertilized eggs (100 %)	1·12	10·8	1·02	0·37	17	109	5·5	8·6	64
Unhardened	1·17	11·9	1·11	0·45	19	104	5·2	8·6	61
	(Mean ± S.D.: 18 ± 1·4.)								
Hardened	1·20	2·40	0·51	0·09	3·8	143	7·2	10·9	66
Normal development (7·5 %)									
Early cleavage	1·17	1·14	0·31	0·035	1·8	143	7·2	9·8	73
Multi-celled blastoderm	1·29	1·25	0·36	0·045	2·2	171	8·6	11·4	76
Beginning epiboly	1·13	1·24	0·31	0·035	1·9	139	7·0	9·7	72
Advanced myomere	1·13	1·58	0·35	0·040	2·2	114	5·7	8·9	64
	(Mean ± S.D.: 2·0 ± 0·2.)								

using D equal to 3·0 and 12×10^{-6} cm^2 sec^{-1}, and determined to be $1{\cdot}8$ and $1{\cdot}6 \times 10^{-5}$ cm sec^{-1}, as compared with the above value of $1{\cdot}7 \times 10^{-5}$ cm sec^{-1}.

There is another important consequence attached to the slow rate of exchange in these teleost eggs, namely that when $L \ll 1$ the influence of unstirred layers upon the value of E is minimal. The mathematical formulation cannot be presented here, but the interested reader can refer to the theoretical papers of Hansson Mild (1971*a*, *b*) in which the affect of unstirred layers has been taken into account by consideration of outside diffusion. To show that this parameter does not change the value of E here reported, a computer program was set up using the experimentally determined changes in RW from one of the experiments with pike eggs. A least-square method was used to obtain the best-fit curve to the experimental points, and the solution for E was calculated (1) by consideration of outside diffusion and (2) without taking outside diffusion into account; the value of E was the same to the first two digits (Hansson Mild, personal communication). Before leaving this point entirely it should be mentioned that although the calculated value of E in the present experiments is relatively unaffected by neglecting outside diffusion, such is not the case with amphibian eggs and embryos since $L \cong 1$ in all cases reported.

The result of complete activation upon L and E can be seen by comparing the data of hardened and unhardened eggs, all of which have been examined in 100% Ringer solution (Table 2). The E value for the former ($3{\cdot}8 \times 10^{-6}$ cm sec^{-1}) is about 4–5 times lower than for the unhardened specimens. At subsequent developmental stages L decreased further, and the exchange coefficient fell to its lowest value.

An example of water exchange in a normally developing egg in early cleavage with its rigid chorion and well-defined perivitelline compartment is shown in Fig. 1C. The time course indicating the completion of water exchange extends over more than 18 h. The perivitelline compartment was estimated to be about 3·0 μl by micrometer. Assuming that nearly all of this volume is water, and that the contents of the perivitelline compartment exchange rapidly, it is expected that the initial increase in reduced weight would be about 60 μg. Inspection of the figure bears out this prediction in a rather precise manner. After renewal of the water phase of the perivitelline compartment ('RW_0' $-RW_1$), the rate of RW changes declined markedly, proceeding exponentially at about $t = 15$ min; $E = 1{\cdot}8 \times 10^{-6}$ cm sec^{-1}, and half-time for the exchange ~ 5 h. The water content by isotope exchange was 73% as determined from the amount of exchangeable water, more conventional methods yielding 79% water volume at the same developmental stage.

Similar exchange curves were obtained for the other normally developing eggs, and these data are also contained in Table 2. In all eggs examined $L \ll 1$; the mean of $E = 2{\cdot}0 \times 10^{-6}$ cm sec^{-1}, with a range of $1{\cdot}8$–$2{\cdot}2 \times 10^{-6}$ cm sec^{-1}. Except for the lower water content obtained at the myomere stage, isotope-exchange measurements indicated a water phase between 72% and 76%, as compared with the average wet–dry determinations of 79%.

Some additional observations of interest to the overall results of this study have also been made and they will be treated here summarily. At the conclusion of each experiment with normally developing eggs (Table 2), the egg was placed in isotope-free dilute Ringer solution and allowed to develop beyond the advanced myomere stage; no abnormalities were observed. In some cases the egg was also reweighed after several

hours in dilute salt solution containing ordinary water; the *RW* of these eggs indicated that the previously accumulated heavy water had been replaced. Under the conditions here imposed these observations show that at the gross anatomical level heavy water does not produce abnormalities, and that during the developmental interval considered egg water can be freely and readily exchanged with the immersion medium.

A graphic illustration of the permeability profile of the developing egg-embryo during the period chosen for analysis is shown in Fig. 2; formalin-treated eggs are included in order to emphasize the removal of their surface resistance to water flow ($E \rightarrow \infty$). While the most striking feature of these data is the marked decline in permeability following egg activation, of equal interest (particularly to our later discussion) is the absolute value of the exchange coefficient of the freshly shed (= unhardened) egg as compared with eggs of similar developmental stages from

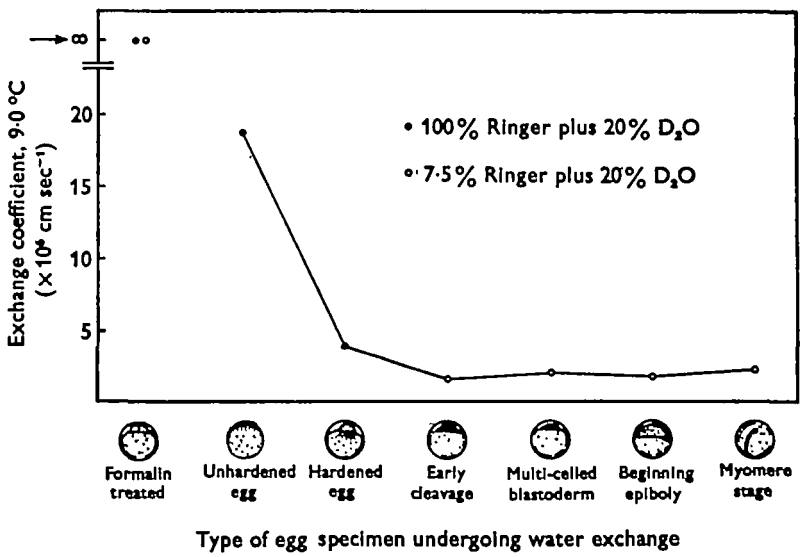

Fig. 2. The permeability of eggs and embryos of the pike, *Esox lucius*, as determined by isotope exchange using the automatic diver-balance.

other species. The pike's mean value, $1{\cdot}8 \times 10^{-5}$ cm sec^{-1}, is found to be nearly identical to the exchange coefficient of unfertilized eggs of the salamander, *Ambystoma mexicanum*, $\sim 2 \times 10^{-5}$ cm sec^{-1} at 8 °C (Haglund & Løvtrup, 1966). Comparable E values at the lower extreme, as observed after activation (= hardened eggs) in the normally developing specimens, have also been reported, $\sim 1 \times 10^{-6}$ cm sec^{-1} at 5·5 °C for the salmon, *Salmo salar*.

The slightly lower E of normally developing eggs, as compared with the hardened egg, could be interpreted as a further 'tightening' of the membrane. However, it might be difficult to support this view as the only influencing factor, for when water exchange was measured in 100% Ringer solution at the same developmental stages from early cleavage to myomere embryos shown in Fig. 2, the values of E were found to range between 4 and 10×10^{-6} cm sec^{-1}. These results are informative in regard to osmoregulation, for they show that the low permeability established after normal egg

tivation can be partially reversed by increasing the tonicity of the immersion medium. Although swelling of these hardened normally developing eggs was not observed during the course of water exchange, continuous exposure to 100% Ringer solution for 20 h or longer resulted in noticeable enlargement of the chorion and the egg proper.

While the significance of these small differences in E between hardened and normally developing egg-embryos is not completely resolved, the data do indicate that the activation process results in a 'membrane' whose coefficient remains of a low order of magnitude up to the myomere stage. The pattern of water exchange in hardened pike eggs is similar to that in salmonid species, a two-phase turnover of water, initially rapid and corresponding to the perivitelline fluid, followed by a protracted exchange of the water in the egg itself. Taken together, these data verify that the view first advanced by Gray (1932) in reference to salmon species also applies to the pike; after egg activation the chorion remains highly permeable to water while the permeability of the structures surrounding the egg proper are reduced.

DISCUSSION

Comparisons between reduced weight changes and wet–dry weight determinations confirmed our previous report and lead to the conclusion that the largest portion of the water phase of the whole egg was exchanged with that of the isotopic medium. The previously accumulated heavy water did not produce abnormal development and could be replaced, once accumulated, with ordinary water after immersion in non-isotopic dilute salt solutions; these observations attest to the continuous water turnover during early development. The diffusion coefficient for water in cytoplasm and the exchange coefficient were found to be similar to those reported for *Salmo salar*. Before turning our attention to these comparative values, let us consider the methodological approach used in the present study to determine D and E.

Methodological considerations. Throughout this presentation we have considered that water exchange could be expressed in terms of the diffusion coefficient, D, and the exchange coefficient, E. The experimental points conformed to the theoretical expectations in the various attempts to obtain estimates of these parameters. One acknowledged difficulty was the possible low estimate of D, since these experiments were conducted without concern for unstirred layers and their affect on the rate of exchange. When such considerations were applied to frog eggs, a two- to threefold increase in D was obtained over those values previously reported (Løvtrup, Hansson Mild & Berglund, 1970). It is not likely that such an increase would apply to the present material, for this would result in a coefficient of about $1{\cdot}2$–$1{\cdot}8 \times 10^{-5}$ cm^2 sec^{-1} at 10 °C, a value slightly greater than the self-diffusion coefficient of water at the same temperature (Kohn, 1965). An alternate approach in order to obtain a comparative estimate of D would be to correct the frog D values from 25 to 10 °C, using Q_{10} data published for *Rana temporaria* (Haglund & Løvtrup, 1966). This gives a diffusion coefficient for ranid species of about 8–9×10^{-6} cm^2 sec^{-1}, similar to the D values of teleost species.

Further discussion will not be given here, but accepting the suggestion of the influence of outside diffusion it can be concluded that whatever subsequent adjustment might be necessary the value of D here reported will be but slightly altered. However,

there are other considerations relating to water exchange and teleost egg structu[illegible] that require additional comment.

In the above theoretical treatment it was implied that the objects undergoing exchange meet certain requirements, such as conformity to a perfect sphere. Upon observing hardened and normally developing eggs, the spherical outline circumscribed by the chorion is apparent, but it is also apparent that the developing egg-embryo is not spherical. Furthermore, beginning with the onset of cleavage, the egg (proper) becomes bounded by a succession of different limiting structures, so that the cell-environmental boundary is no longer composed of the same continuous, uninterrupted membrane. These observations lead to further problems, for although it has been assumed that these boundary structures are not differentially permeable, both rate and path of water flow are also unresolved in the present circumstances.

Nevertheless, it seems that the requirements imposed by theory are sufficiently well met in the present material; for example, the outline of the developing egg proper is approximately spherical during the interval studied. Variations in diameter were not observed during the water-exchange interval, so that the average values of R used in these determinations can be considered to serve as reasonable evaluations of egg size. As for the cell boundary, our indices of the surface resistance to water flow (E) can be considered as an average measurement relating to the different morphological structures that surround the egg proper. Until an approach is available to take the above refinements into consideration, our measurements can be accepted as close approximations of the exchange coefficient.

Mechanisms involved in osmoregulation or water balance. While these results show that osmoregulation does not depend upon the impermeability of the cell surface, the question remains as to the factors that do function to inhibit uncontrolled flooding as these eggs develop in their natural environment. In other teleost species it has been suggested that the main factors responsible for the prevention of osmotic swelling are the combined effects of the colloidal material within the perivitelline compartment and the mechanical properties of the chorion (Bogucki, 1930; Kao & Chambers, 1954). The importance of these factors can also be gathered from those observations (p. 800) made in the present study; when freshly shed eggs were kept in Ringer solution for 6 h or longer both the perivitelline space and the egg proper increased in volume. In the case of the chorion this swelling continued, until the perivitelline compartment had increased to about twice the volume observed in normally activated eggs. Such volume increases did not take place in the experiments with hardened specimens over the same time interval, and it therefore seems reasonable to conclude that when the chorion is hardened the pressure in the perivitelline compartment, maintained by water uptake in response to the colloidal material within, is instrumental in preventing egg enlargement.

But the above observations also imply that there are other factors to be considered in osmoregulation, namely (1) the mechanical properties of the investing cell layers of the egg (plus the egg cortex), and (2) the rate of water exchange across these structures. The relation between these factors can be judged from the experiments with hardened normally developing eggs tested in Ringer solution (p. 804) and those determinations with unhardened eggs (Table 2). In each experimental series swelling was detected after 20 and 6 h immersion in 100% Ringer and in each series permeability was

measureably higher than that of normally developing eggs in dilute salt solution, slightly in the case of the hardened eggs in Ringer medium ($4–10 \times 10^{-6}$ cm sec^{-1}) and considerably ($1{\cdot}7$ and $1{\cdot}9 \times 10^{-5}$ cm sec^{-1}) in those unhardened eggs (Table 2).

It is difficult to dissect this phenomenon further – to separate cause and effect – but it seems that the ionic concentration of full-strength Ringer is sufficient to suppress chorionic hardening and to alter the physical properties of both the hardened chorion and the membranes that surround the egg itself. As denoted by the easily deformable chorion, the measured increase in size and the exchange coefficient, these changes were expressed in terms of (1) a decreased hydrostatic pressure, (2) enlargement of the whole egg and the egg proper, and (3) increased permeability to water. The opposite situation applies in normal activation: the volumes of the egg and the perivitelline compartment are stabilized, together with an increase in hydrostatic pressure and decrease in permeability. These changes, together with the mechanical properties of the membranes, act in conjunction to maintain water balance within acceptable limits – even if during normal activation they cannot prevent a slight enlargement – and thus are the controlling elements in osmoregulation. In view of the importance of the activation process in teleost eggs, as well as of the fact they are water permeable, it would seem desirable to compare their exchange coefficients with those reported for other species in an effort to determine whether other indications can be obtained.

Comparative values of the exchange coefficients of eggs and embryos in certain teleost and amphibian species. Although a large amount of data is available, permeability studies are unfortunately often conducted under widely divergent conditions – both experimental and theoretical – so that meaningful comparisons are difficult to obtain. Such factors as (1) stage of development (Løvtrup, 1960), (2) tonicity of the test medium (Berntsson, Haglund & Løvtrup, 1964), (3) temperature (Haglund & Løvtrup, 1966) and (4) unstirred layers (Hansson Mild, 1971*a*, *b*) have been reported to influence the value of the coefficients in amphibian egg-cells –similar indications have been observed in the present study. Our discussion will therefore be restricted to reports in which the experimental conditions and theoretical assumptions are similar, attempting at the same time to standardize these comparisons by the following considerations.

The calculations and recalculations (Løvtrup, 1960; Potts & Rudy, 1969; Prescott & Zeuthen, 1953) of E have been determined by the same method (Løvtrup, 1963) and plotted as logarithms according to egg type or developmental stage (Fig. 3). To ensure that tonicity influences have been excluded, only those experiments conducted under isotonic conditions have been chosen. The values of E have been taken from the papers of Haglund and Løvtrup cited in the bibliography and from the references above; data pertaining to *Triturus pyrrhogaster*, *Xenopus laevis* (body-cavity eggs) and *Salmo salar* (gastrula-myomere stages) are unpublished results of the author. For ease of visualization, the species investigated at several stages have been interconnected and the first letter of the generic name placed at the mid-point of the reported range; single determinations are indicated by letter only. All determinations have been conducted within a temperature range of 20–24 °C, except for *Esox lucius* (9·0 °C) and *Salmo salar* (3–5 °C). Evaluation of differences in E is made difficult by such large temperature differences, and we have attempted to compensate for this by adjusting the reported experimental range of the ranid species to 10 °C (dashed lines) using Q_{10} values published by Haglund & Løvtrup (1966) for amphibian species. This

expedient permits comparisons within a range of about 5 °C, and has the additional advantage (except for the tropical zebra fish, *Danio rerio* and the frog, *Xenopus laevis*) of comparing permeability at temperatures that closely approximate those encountered by the developing organisms in their natural environment. Concerning unstirred layers the listed E values have been determined without regard to this parameter. Previous mention has been made in the case of slow exchange in *Esox*; as far as the remaining species are concerned it can be shown that when the exchange coefficient is recalculated with higher values of D, an elevated E value of the same order of magnitude is obtained. Thus such adjustments that might arise from the use of higher D values for the diffusion coefficient would not detract from the general impression that can be gathered from the present data.

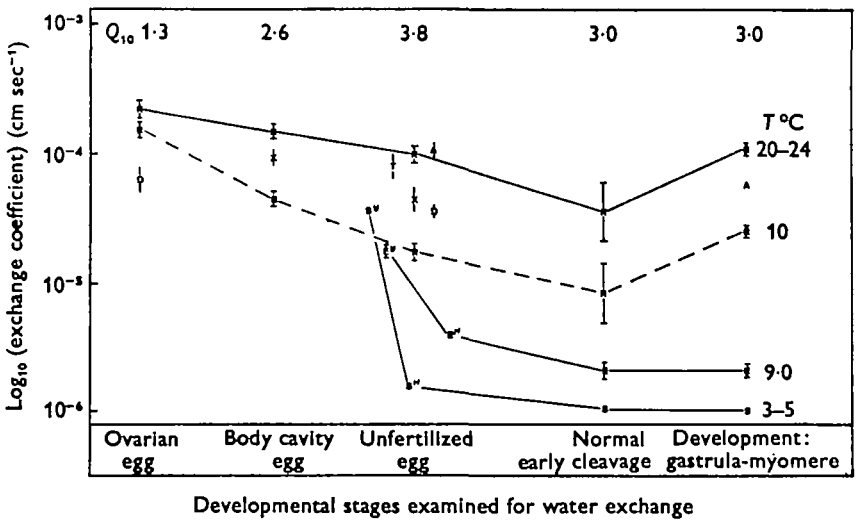

Fig. 3. Comparisons of the exchange coefficients of eggs and embryos from certain amphibian and teleost species as determined by isotope exchange, at the temperatures indicated. Vertical lines denote the reported range of E; single determinations are indicated by letter. Ranid species have been corrected for temperature from 22–24 °C (solid lines) to 10 °C (dashed lines), using the Q_{10} factors shown above each developmental 'stage'. The letter symbols represent: *A*, *Ambystoma mexicanum*; *D*, *Danio rerio*, the zebra fish; *E*, E^U, E^H, hardened normally developing, unhardened and hardened eggs of *Esox lucius*; *R*, the ranid species *esculenta*, *pipiens* and *temporaria*; *S*, S^U, S^H, normally developing, unhardened and hardened eggs of *Salmo salar*; *T*, *Triturus pyrrhogaster*; *X*, *Xenopus laevis*.

Turning our attention to these comparisons, it is apparent that permeability to water, as denoted by the logarithm of the exchange coefficient, tends to decrease as development progresses. The decline in permeability following exposure to the environment is precipitous in the case of *Esox* and *Salmo*, and the permeability remains of a low order of magnitude throughout their early development. Elevated E values at the most advanced amphibian stages may be related to the large fluid-filled cavities present in these embryos (Haglund & Løvtrup, 1966), but absent in developing teleost species.

Although it can be shown that significant differences do exist between many of the listed E values, it is rather surprising to find, in spite of the variation in temperature,

that the exchange coefficients of these species are similar both within and between the different egg classes. Omitting the unfertilized hardened and normally developing stages of *Esox* and *Salmo*, the exchange coefficient covers an order of magnitude, from about $1{\cdot}7 \times 10^{-5}$ cm sec^{-1} to $2{\cdot}5 \times 10^{-4}$ cm sec^{-1}, in the developmental interval from ovarian egg to myomere stage. In general, these data support the idea advanced by Dick (1959) that the permeability (= exchange) coefficients of small cells are higher than those of larger cells. However, the correlation is not absolute, for without exception the E values of *Danio* and *Xenopus* are consistently lower than those listed for urodele and ranid species, all of which have diameters that are greater than those for either *Danio* or *Xenopus*.

After applying temperature corrections to the ranid species the values of E converge toward those of *Esox* and *Salmo*; similar stage-dependent corrections could also be applied to the other species, but they have been avoided here. The similarities in E are most marked in the unfertilized egg, which is, paradoxically, also the point of departure following the activation process in the teleost species mentioned. It should be re-emphasized that it would be misleading to attempt to regard the highly pressurized perivitelline compartment or the rigid chorion as the sole factors responsible for the decrease in permeability following egg activation in *Esox* or *Salmo*; direct evidence for the importance of the entire activation process has been given above. Further support for the aforementioned view can be gained from the following comparative considerations based upon the E values of unfertilized eggs of *Triturus pyrrhogaster* and *Ambystoma mexicanum*. Eggs of the former species possess a highly pressurized perivitelline compartment and a rigid, thick chorion (as in the eggs of pike and salmon); both structures are present in the axolotl but the extraembryonic compartment is not pressurized, nor is the chorion a thick rigid structure. The values of E shown here have been obtained with the chorion intact (T) and removed (A); the similarity between the uncorrected E values of these two urodele eggs, and their difference from the exchange coefficients of the hardened eggs of *Esox* (E^H) and *Salmo* (S^H), does not support the notion that hydrostatic pressure and hardened chorion are the factors solely responsible for the decreased permeability of these teleost eggs. Such comparisons tend rather to support the orthodox viewpoint as recently stated by Potts & Rudy (1969) when referring to *Salmo*: the net effect of egg activation is an alteration in the permeability of the limiting egg membrane. The slow rate of water exchange that is maintained throughout development in *Esox* and *Salmo* is accounted for by their relatively large egg size, the low temperature and the depressed permeability of the different membranes that successively surround the egg proper, rather than by an altered physical state of the egg-bound water (Zotin, 1965).

Whatever degree of 'tightness' is to be assigned to the membranes of *Esox* and *Salmo*, it must be admitted that their coefficients become less than those of the frogs and salamanders here considered. Their reduced permeability is the consequence of an alteration proceeding from the activation process triggered by the hypotonic environment. Yet, in spite of their lower permeability, it is to be noted that their exchange coefficients are not greatly different from those of amphibian species – egg-cells commonly recognized as being freely permeable to water.

SUMMARY

1. The amount and the rate of exchangeable water was determined in cell analogues and normally developing egg-embryos of the pike within the developmental interval from egg shedding to advanced myomere embryos using the automatic diver-balance.

2. Comparisons between the volume of water exchanged and the percentage water content obtained by wet–dry weight determinations confirmed the view that essentially the entire water content of the whole egg – chemically treated and untreated – was exchanged with the isotopic medium. When developing specimens were placed in isotope-free media at the conclusion of the water exchange experiment, they readily exchanged the accumulated isotope for ordinary water and continued to develop normally. On the basis of the foregoing observations, it was concluded that, during the embryological period under investigation, the developing organism undergoes a continuous cyclic water turnover with the environment.

3. Water exchange in chemically treated eggs (= cell analogues) proceeded rapidly, in one continuous uninterrupted phase indicative of a diffusion process in the absence of a surface barrier. The diffusion coefficient, uncorrected for the possible affect of unstirred layers, was about the same as *Salmo salar*: 6×10^{-6} cm^2 sec^{-1}, at 9·0 °C.

4. The pattern of water exchange in untreated eggs was distinctly different than that of the treated specimens, proceeding in a two-step manner: (1) a rapid, initial exchange of the fluid-filled perivitelline compartment (2) followed by a prolonged exchange of the egg proper which was characteristic of a diffusion process in the presence of a surface restriction to water flow. The exchange coefficient of unhardened eggs, immersed in Ringer solution to inhibit chorionic hardening, was considerably higher ($1{\cdot}8 \times 10^{-5}$ cm sec^{-1}) than the hardened specimens ($2\text{–}4 \times 10^{-6}$ cm sec^{-1}).

5. Additional observations of the affect of Ringer solution upon egg 'swelling' and the exchange coefficient strongly support the view that the total activation process is vital to maintaining the proper water balance. It has been suggested, in conformity with the observations of previous investigators, that the consequence of the activation process results in an alteration of the permeability characteristics of the membrane surrounding the egg proper.

6. The exchange coefficients of eggs and embryos from several teleost and amphibian species were compared at a number of similar developmental stages: it was observed that there is a general tendency for the exchange coefficient to decrease as development progresses. Although significant differences can be shown between the exchange coefficients of different species, as well as between stages within the same species, the values of E were found to occupy a rather restricted range: corrections for temperature reduced this range further so that there was little separation between many of the recorded values. The exceptions to this general 'rule' were the exchange coefficients of hardened eggs and embryos of *Esox* and *Salmo*, which were less than those of all other species. Yet, the depressed value of E in *Esox* and *Salmo* is not greatly different from those of amphibian egg-cells which are commonly recognized as being freely permeable to water.

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