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Embryology

NEW DATA ON THE MECHANISM OF WATER UPTAKE IN SALMONID EGGS

NOWE DANE DOTYCZĄCE MECHANIZMU WCHŁANIANIA WODY PRZEZ JAJA RYB ŁOSOSIOWATYCH

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Water uptake of eggs of *Salmo trutta* L. and *Salmo gairdneri* Rich. in water and 1/15 M buffers was studied. It was found that if buffers prevent the formation of the perivitelline space in the eggs placed in them immediately after spawning, they do not exclude its normal formation in the eggs placed for 2 or even 1 minutes in water prior to their being transferred to a buffer.

The authors express their opinion that the water uptake of eggs proceeds in two stages and that there are correspondingly two factors, an osmotic and a colloidal, responsible for this process.

The problem of water uptake in the fish egg directly following its fertilization and, consequently, that of the mechanism of formation of the perivitelline space have recevied very much attention and yet they have not been solved definitively till now. All the authors agree that immediately after an egg has been placed in water, the water enters it by the micropyle and penetrates through its membrane, which originally is permeable to water and salt (**Bogucki**, 1930; **Manery** and **Irving**, 1935; **Kanoh**, 1950, 1952; **Zotin**, 1961), but divergencies arise when it comes to the explanation of the mechanism of water uptake and to the manifestation of the forces responsible for it.

Bogucki (1930) put forward a hypothesis that both the penetration of water and its amount taken up by the egg depend in the first place on the amount of hydrophilous colloids in the egg, which takes up water until they have been saturated.

Needham (1931), Hayes (1949) and Hayes and Armstrong (1942) hold similar views. Many authors however, among them Yamamoto (1939), Ito (1960) and Zotin (1961), give special attention to the role of osmotic activity of the egg in the process of water uptake.

The present study is an attempt to explain the still controversial role of osmotic pressure inside the egg in the process of water uptake and in the formation of the perivitelline space.

MATERIAL AND METHOD

This investigation was carried out in the Institute of Ichthyology, Academy of Agriculture, at Szczecin in November-December 1971 and March-April 1972.

Eggs of the sea-trout (*Salmo trutta* L.) caught in the River Reda and rainbow trout (*Salmo gairdneri* Rich.) from the pond fish culture of the Regional River Laboratory, Institute of Inland Fisheries, at Gdańsk-Oliwa were used as study material.

Sexual products of the fishes (eggs and sperm) were placed in vacuum flasks immediately after spawning on the spot and transported dry to the laboratory at Szczecin, where the present experiments were carried out. Although earlier studies showed that the long transport of „dry" eggs and sperm has no major influence upon the initial stages of embryonic development, two control cultures in water were laid down, one immediately after spawning and the other 12 hour later, when the eggs had arrived in the laboratory, in order to determine the action of transport, if there was any, on the results.

Experiments were started 12 hours after spawning. They consisted in observing the changes in the turgor and those in the strength of the membrane of the eggs which had been kept in water for 1, 2, 3, 5, 10 and 20 minutes before their being transferred to buffers and incubated in them. Some of the eggs (control 3) were placed in buffers directly after having been mixed with sperm. The buffer used in the study was 1/15 M Michaelis's phosphate buffer at pH 5.5, 7.1 and 8.5. The turgor of eggs was determined 60 minutes after fertilization and next everyday for 5 days and the strength of egg membranes was measured everyday starting 24 hours after fertilization.

Changes in the turgor of eggs, as indirect indications of the rate of water uptake, were determined by an objective graphic method (Winnicki, 1967) and the strength of egg membranes was measured by the modified Schäperclaus's method (1940).

All the eggs were incubated in liquids (water and buffers) in crystallizers placed in a water jacket at 11–12°C.

RESULTS

The present experiments show that eggs, spawned and placed in buffers, irrespective of the pH of these last, do not take up water, their turgor does not increase at all, the

perivitelline space does not appear and no increase is observed in the strength of egg membranes, whereas the eggs placed in water prior to their being transferred to buffers take up water, their perivitelline space comes into being, the embryonic plate is formed and the strength of egg membranes also increases. These changes are not all uniform, being dependent on a) the kind of buffer and b) the length of the stay of eggs in water preceding their transfer to a buffer.

The results obtained are illustrated in a diagram constructed of kymograms characterizing the turgor of eggs transferred from water to buffers at different times (Fig. 1) and in a table, in which the mean values of the strength of these eggs 12 hours after the commencement of the experiment are presented (Table 1).

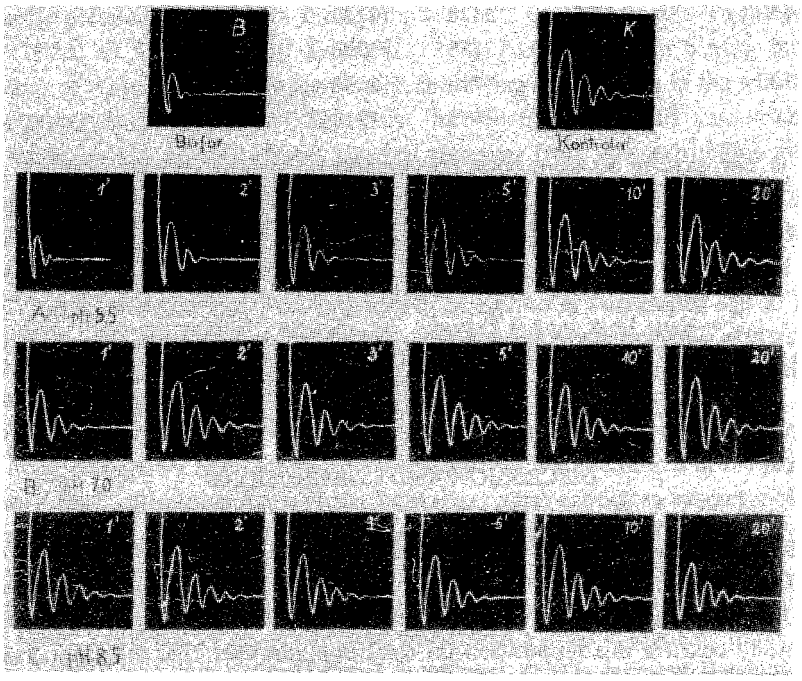

Fig. 1. Turgor of trout eggs transferred from water to buffers at various pH, determined 60 minutes after fertilization (B and K – controls in a buffer and water; 1'..... 20' – length of stay of eggs in water before their being transfered to buffer)

The investigation carried out showed that the placement of eggs in water for 2, 3, 5, 10 and 20 minutes before they are put for good in buffers ensures the uptake of water from the surroundings. It is obvious that this process runs the more efficiently the longer the egg has stayed in water and is slowed down in the cases in which the egg has been kept in water for hardly 2, 3 or 5 minutes, and this is especially true of the fluids at pH 5.5. The final effect was, however, always the same: sooner or later the experimental eggs attained the same turgor as the control eggs incubated in water. Moreover, eggs placed in water for only 1 minute, took up water from environment in buffers at pH 7.0

and 8.5. In this case the only exception were the eggs transferred to a buffer at pH 5.5, no increase in their turgor being observed.

Table 1

Strength (g) of membranes of trout eggs placed for given lengths of time in water prior to their incubation in buffers. Measurements taken 12 hours after fertilization.

Time of stay in water (in minutes)	pH 5.5	pH 7.1	pH 8.5	Control	
				Buffer	Water
1	260	1060	690	240	1430
2	370	2950	1080	–	–
3	1730	3420	1125	–	–
5	3790	3535	1200	–	–
10	3270	3740	1280	–	–
20	3500	3310	1310	–	–

This is not exactly so as regards the strength of egg membranes. Table 1 suggests a certain dependence of the strength (measured 12 hours after fertilization) on the length of stay of the eggs in water prior to their incubation in buffers. This was particularly well seen in the case of the buffer at pH 5.5. Another noteworthy phenomenon is that the strength of egg membranes in buffers at pH 5.5 (from 5 minutes upwards) and 7.0 (from 2 minutes upwards) was about twice as high as that in water controls, whereas in the eggs placed in buffers at pH 8.5 it lay within the limits of the strength of control eggs.

DISCUSSION AND CONCLUSIONS

A supposition was expressed in the previous paper (Cykowska and Winnicki, 1972) that eggs can take up water from the environment of a higher osmotic pressure than that prevailing in the eggs, or strictly in the perivitelline fluid. The results obtained in the present study confirm this supposition on condition, however, that the egg stays initially in water for, at least, 1 to 2 minutes at a temperature of 12°C and therefore for a much shorter time than that during which the egg takes up water under normal conditions. According to Zotin (1955), this last time is about 50 minutes under given conditions, whereas in the period of 2 minutes the egg has hardly enough time to take up 3–5% of the amount of water it receives from the environment during its swelling.

What therefore causes that the egg placed in a liquid of a high osmotic concentration immediately after spawning does not take up water, although when placed in the same environment after the process of uptake has been activated, it receives water which permeates in the opposite direction to that determined by the differences in molecular concentrations?

The answer to this question would be simple, if, as suggested by Aoki (1942), there were an amount of colloidal substances in the perivitelline gap, i.e., between the perivitelline membrane and the egg membrane; after the contact of the egg with water these

substances would cause the permeation of water through the egg membrane and next, owing to the differences in osmotic concentrations, the bursting of the cortical alveoli, the contents of which contributes to and accelerates the process of water uptake on the one hand, and induces changes in the membrane itself on the other hand (**Zotin**, 1953). However, this is not the case, for **Kanoh** (1950) demonstrated clearly that at the time when eggs are laid into water there is not a trace of colloids, even between the egg and perivitelline membranes.

It might also be supposed that at the moment when the egg is laid into water, when there is no perivitelline space and the egg membrane adheres closely to the perivitelline membrane, the whole of the egg behaves as a sort of osmotic aggregate, owing to which the osmotic pressure prevailing in the deutoplasm and, as will be seen from the papers by **Svetlov** (1928) an **Klekowski** and **Domurat** (1967), coming to about 6 Atm, causes the permeation of water through the membrane, thus being the first link in the whole mechanism of water uptake. The first link only, for when even a small amount of water permeates under the egg membrane, this last separates from the perivitelline membrane and the initial osmotic aggregate stops working, as then the deutoplasm is already separated from the ambient environment by two semi-permeable membranes and a layer of liquid of low osmotic properties between them.

However, this hypothesis must also be rejected, for if it were true, the egg placed in an environment with an osmotic pressure of about 1.5 Atm, and such was the osmotic pressure produced by buffers used in our experiments, would have to take up water, since the osmotic pressure of the deutoplasm, as given above, is about 4 times as high.

Having excluded the two foregoing possibilities, we should accept that being laid in water the egg contains a slight amount of osmotically active substances in the perivitelline gap. The osmotic pressure generated by them is not great enough to extract molecules of water from an environment with a great molecular concentration. However, under water conditions, normal for the ecology of the species, it is suffiently high for the water to permeate under the membrane. Thus, when the egg stays in water, even for a short time (in our experiments for 1–2 minutes) and when even a small amount of water gets into the perivitelline gap, the cortical alveoli burst and at this moment the hydrophilous colloids, released from the alveoli, undertake the role of the main pump sucking water from the environment. The activation of the colloidal „suction pump" causes the permeation of water through the egg membrane in the direction opposite to that determined by differences in the osmotic concentrations. The process of water uptake comes to an end, when the egg membrane, which determines the size and volume of the egg and is folded in an unfertilized egg (**Winnicki** et al., 1968), has become stretched, which is indicated indirectly also by the fixed turgor of the egg (**Winnicki**, 1967).

The slowing-down of the process of water uptake in an environment with a pH of 5.5 shows that the movement of water through the egg membranes is determined, at least to the same extent as by physical mechanisms, by other factors, above all, enzymic ones. This theory is supported by both the data concerning the inhibitors of esterases responsible for the breakdown of the cortical alveoli published by Japanese investigators

(Yamamoto, 1951; Hamano, 1951; Kanoh and Yanagimachi, 1956) and the very fact that in our experiments the strength of the membranes of eggs incubated in buffers at pH 5.5 and 7.0 was twice as great (and consequently less permeable), which indicates the active share of the membrane-hardening enzyme in this process.

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NOWE DANE DOTYCZĄCE MECHANIZMU WCHŁANIANIA WODY PRZEZ JAJA RYB ŁOSOSIOWATYCH

Streszczenie

Badano wchłanianie wody przez jaja *Salmo trutta L.* i *Salmo gairdneri* Rich. w wodzie i w płynach buforowych o molarności 1/15 M.

Stwierdzono, że o ile płyny buforowe uniemożliwiają powstawanie przestrzeni periwitelarnej w przypadku, kiedy jaja umieszczone są w nich tuż po tarle, o tyle uprzednie 2-, a nawet 1-minutowe przetrzymywanie jaj w wodzie z następnym przeniesieniem ich do buforów nie wyklucza powstania normalnej przestrzeni okołożółtkowej.

Autorzy formułują pogląd o dwuetapowości wchłaniania wody przez jajo i odpowiednio o dwu czynnikach, które o tym procesie decydują – osmotycznym i molekularnym.

НОВЫЕ ДАННЫЕ КАСАЮЩИЕСЯ МЕХАНИЗМА ПОТРЕБЛЕНИЯ ВОДЫ ЯЙЦАМИ ЛОСОСЕВЫХ РЫБ

Р е з ю м е

Изучали потребление воды яйцами Salmo trutta L. i Salmo gairdneri Rich. в воде и буферных жидкостях - 1/15 М.

Нашли, что если данные буфера полностью затормаживают возникновение перивителлинового пространства в случае, когда яйца помещены в них сразу после нереста, то 2 и даже 1-минутная выдержка яиц в воде и следующее затем погружение этих яиц в буфере не исключает возникновения нормального перивителлинового пространства.

Авторы формулируют тезис р двухэтапности засасывания воды яйцами и сообразно тому о двух факторах, которые в этом процессе участвуют - осмотическом и коллоидном.

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