Hardening of the Chorion of Salmon Egg¹

By

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I

In the eggs of many kinds of fish, fertilization is usually accompanied by elevation of the chorion, as in the case of the fertilization membrane of the sea urchin egg. However, in the unfertilized salmon egg, the elevation of the chorion occurs by absorbing water, when immersed. On the mechanism of the water absorption in the salmon egg, Aoki has reported in a series of papers ('39, '40 and '42). According to him, water absorption generally reaches equilibrium within two hours in tap water or other hypotonic solutions. The degree of water absorption increases with the decreasing osmotic concentration of the medium (initial water absorption) ('39). When the egg, which has already fully absorbed water, is dehydrated in hypertonic colloidal solution, a depression appears on the chorion, as in the case of shrinkage of the chorion of the *Oryzias* egg (Yamamoto '36). The depression does not disappear as long as the egg is kept in the solution. The eggs so dehydrated absorb water rapidly not only in the water or hypotonic solution but also in hypertonic solution and approximately recover their initial state (water re-absorption); the final weight becomes, on the average, about two per cent less than that of the initial weight (Aoki '42). The chorion of the fresh water-un-absorbed egg is soft and delicate, but it hardens and becomes very tough when the eggs are brought into contact with water, as has been observed and reported by some authors. As a consequence of this hardening, the equilibrium state of the water absorption of the salmon egg may be affected by the maximum elasticity of the outer membrane, the "chorion", consequently, the equilibrium degree of water absorption can be used as a measure of degree of the hardening of the chorion. The depression at the time of dehydration and lowered degree of water re-absorption are perhaps related to the hardening. In order to ascertain the relationship between hardening of the chorion and the degree of water absorption, the following experiment was made complementally.

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II

The fresh unfertilized eggs obtained directly from the oviduct of the salmon (*Oncorhynchus keta*, Wahrburg) were employed as the material. The procedures for the initial absorption and re-absorption of water were done almost in the same way as those made by Aoki ('39, '40 and '42). However in this study the dehydration was made in 6% solution of gum arabic rather than 10% solution. The temperature was kept constant at $9.0^{\circ}\text{C} \pm 0.5$ during the experiment, and the pH of both re-distilled water and balanced salts solution (b.s.s.)² was regulated as ca 7.0 by adding 1/10 N sodiumbicarbonate. About ten eggs were employed in each case to measure weight. The degree of water absorption in the following tables represents the increase of egg weight resulting from water absorption expressed in per cent.

III

The degree of water re-absorption in tap water is influenced by the duration of time of the initial water absorption in tap water (Table 1), that is, it increases proportionally to the length of time of the initial water absorption. As shown by Aoki ('39 and '42), the preparation period for the water absorption in the egg is completed within about 10 minutes after immersion in water. This may be the case, so far as the preparation of initial water absorption is concerned, in the above experiment. Therefore the difference in the degrees of re-absorption as shown in the Table 2 is hardly ascribable to the change of the colloid

Table 1. Relation between increase of egg weight resulted from re-absorption of water and duration of initial water absorption in tap water

Duration of initial absorption in tap water in minutes	Increase of the egg weight after re-absorption in tap water in %
10	12.4 ± 0.08
30	14.5 ± 0.09
60	15.1 ± 0.06
120	15.9 ± 0.11

osmotic force of the perivitelline fluid. Here it must be noticed that the equilibrium state of the water absorption is also affected by the maximum elasticity of the chorion. After coming into contact with water, the chorion becomes somewhat opaque in colour and hardens losing its elasticity. Assuming that the chorion hardens after having enlarged as

² As to the component of b. s. s., refer to Aoki '39.

³ It may be the peptisation of the gelatinous substances in the perivitelline space.

a result of the initial water absorption, the degree of the re-absorption of water must differ in accordance with the grade of distensibility, the grade corresponding to the degree of initial water absorption. From these considerations, it may be concluded that, the chorion hardens during the initial water absorption in tap water and also during the dehydration in the solution of gum arabic.

If it is true that the chorion hardens by water absorption in tap water, the following hypothesis may be made: when the water absorption in tap water is interrupted before reaching equilibrium, further absorption of water would be influenced by the duration of interruption. To test this hypothesis, the following experiment was made. The eggs, which had previously absorbed water for ten minutes in tap water, were immersed in liquid paraffine for different intervals. Then, by transfer into tap water, the water absorption was resumed and continued until equilibrium was attained. As Fig. 1 indicates, the longer the immersion time in liquid paraffine, the lower the degree of equilibrium absorption of water.

Fig. 1. Effect of the interruption of initial water absorption on the further absorption

The fresh salmon egg can neither absorb water in isotonic b.s.s. (1/8) nor in hypertonic gum arabic solution (10%). However, the egg which has been treated in gum arabic solution previously can absorb water even in isotonic b.s.s. After the immersion in gum arabic solution the chorion becomes somewhat opaque in colour precisely like that of water absorbed eggs. This indicates that the chorion imbibes water in

hypertonic solution of gum arabic and that in addition some changes occur on the colloidal state of the perivitelline space. As is shown in Table 2, the longer the immersion time in solution of gum arabic, the less the absorption in 1/8 b.s.s.. It can be assumed that the chorion hardens

Table 2. Water absorption in 1/8 b.s.s. of the eggs which have been immersed in 10% solution of gum arabic in different durations

Duration of immersion in gum arabic in minutes	Degree of water absorption in 1/8 b. s. s.
0	0.02 ± 0.03
10	5.2 ± 0.09
60	4.9 ± 0.09
120	3.2 ± 0.04

even in solution of gum arabic, and that, as the hardening velocity is slow, the degree of water absorption gradually decreases, proportionally to the duration of the time of the immersion time.

As shown in Table 2, the chorion hardens in solution of gum arabic, and this satisfactorily explains the result in Table 3.

Table 3. Relation between increase of egg weight resulted from re-absorption and duration of initial absorption in re-distilled water

Duration of initial absorption in re- distilled water in minutes	Increase of the egg weight after the re- absorption in re-distilled water in %
10	18.8 ± 0.13
30	21.7 ± 0.21
60	22.8 ± 0.14
120	23.6 ± 0.17

Here it is shown that the chorion hardens in the course of dehydration of the enlarged one, and this yields the difference in the degree of re-absorption in a similar way even in the medium of re-distilled water

Table 4. Comparison of the degrees of re-absorption and those of re-re-absorption in tap water

Duration of initial water absorption in minutes	Degree of re-absorption	Degree of re-re-absorption
10	12.4 ± 0.08	12.9 ± 0.13
30	14.5 ± 0.09	14.3 ± 0.10
60	15.1 ± 0.16	15.5 ± 0.18
120	15.9 ± 0.11	15.8 ± 0.11

If the hardening of the chorion is irreversible, the chorion will remain fixed in that shape once hardening is completed. Then, even though the eggs are dehydrated in hypertonic colloidal solution, the state of the chorion can no longer be reversed. Consequently, the degree of re-re-absorption of water may be equal to that of re-absorption, this hypothesis being verified in Table 4.

IV

It has been found in the ova of the sea urchin, that hardening of the fertilization membrane depends on the existence of bivalent cations in the surrounding medium. As is apparent in the experimental results shown in the above paragraph, the hardening of the chorion of salmon egg takes place in tap water and also in solution of gum arabic; in the former bivalent cations are present while the main components in the latter are Ca and Mg salts of arabic acid. In these respects, there is a close similarity between the hardening of the fertilization membrane and that of the chorion.

The degree of water absorption in b.s.s. increases with decreasing salts concentration (Aoki '39). When the eggs which have absorbed water fully in the different concentration of b.s.s., are transferred into

Table 5. Degree of continuous water absorption in tap water of the eggs which have fully absorbed different concentrations of b.s.s.

Concentration of the b. s. s.	Degree of continuous water absorption in tap water
1/8	13.9 $\pm$ 0.09
1/16	13.9 $\pm$ 0.08
1/64	13.7 $\pm$ 0.12
1/256	15.0 $\pm$ 0.16

Table 6. Degree of re-absorption of water in tap water of the eggs which have previously been equilibrated in different concentrations of b. s. s.

Concentration of the b. s. s.	Degree of re-absorption of water in tap water
1/16	2.0 $\pm$ 0.16
1/32	11.2 $\pm$ 0.15
1/64	13.3 $\pm$ 0.05
1/256	15.5 $\pm$ 0.13

tap water, the degrees of the following water absorption are nearly equal, independently of the concentration of b.s.s. used (Table 5). But

there exist marked differences in the degrees of re-absorption of water (that is, water absorption after the dehydration), varying with the concentration of b.s.s. in which the eggs have been treated previously (Table 6). These two facts suggest that some change may occur during the immersion in solution of gum arabic and this change may cause the hardening of the chorion. The b.s.s. undoubtedly contains bivalent cations. The existence of bivalent cations in b.s.s. is without doubt responsible for the hardening reaction. From the results in Table 5, however, no hardening of the chorion seems to occur, even in the b.s.s. Thus, it may be suggested that the hardening of the chorion not only depends on the existence of bivalent cations in the surrounding medium, but some other factor, perhaps the grade of hypotonicity of the medium, also takes a part in this hardening. Further study may furnish an exact explanation for the hardening of the chorion.

When a strip of the chorion of salmon egg which has not brought in contact with water, is immersed in acid (1/100 N HCl), the inner layer of the chorion swells strikingly at first, and then disperses gradually. But this swelling phenomenon in acid is completely absent after long immersion in rather hypotonic b.s.s. or in tap water. Further, the chorion which has been treated in isotonic CaCl_2 solution swells very little in acid, while in one treated with isotonic NaCl solution considerable swelling occurs (Aoki '41). These facts suggest that contact with water containing Ca ions produces an irreversible change in the chorion.

From the facts cited above, it may be concluded that bivalent cations play an important rôle in the irreversible changes of the chorion, such as hardening, as well as the loss of the swelling property in acid, which take place with water absorption of the egg.

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Summary

The hardening of the chorion of salmon egg has been studied.

1) The degree of re-absorption of water depends upon the duration of initial absorption in tap water, and in re-distilled water.

2) The degree of later absorption of water differs according to the duration of interruption of water absorption when liquid paraffine is employed.

3) The degree of water absorption of the eggs in isotonic b.s.s. which have been immersed in 10% solution of gum arabic, differs accord-

ing to the duration of immersion.

4) Hardening is an irreversible change.

5) From these facts, it may be concluded that the hardening of the chorion is caused not only by the existence of bivalent cations in the surrounding medium, but also by some other factor, which has not been clarified. It is probable that the tonicity of the medium plays a rôle in the hardening reaction.

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