# A Brief Note on the Permeation of Heavy Water into the Unactivated Eggs of the Raindow Trout[1]

By

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Some fifteen years ago, the permeability of eggs to heavy water was under study by Krogh and Ussing (1937), utilizing unfertilized eggs of the rainbow trout, *Salmo irideus*. In thier investigation, the unfertilized eggs were immersed in dilute heavy water ; but, as will be stated later, such salt-free media have been found to give rise to the activation of eggs (Kusa, 1950). It is, therefore, still uncertain whether the actual permeation of heavy water takes place with or without the activation of the eggs. In order to determine this question, this investigation was undertaken with unactivated eggs of the same trout species.

The experiment was performed using principles introduced by Krogh and Ussing, except that 1.82 per cent heavy water in the isotonic Ringer[2] was employed as an experimental medium. It is known that in the isotonic Ringer, both with and without such a small quantity of heavy water, activation of the unfertilized eggs is reversibly inhibited, and no perivitelline space is formed, as in the salmon eggs in the isotonic Ringer (Kusa, 1950). Further, no harmful effect of the solution is detected in the eggs even after 24 hours of immersion. The eggs (ca. 10 grms), squeesed out from the adult female, were immediately placed in 10 cc. of the heavy-water Ringer in a vessel, covered with a lid, to prevent evaporation. A sample of the medium was taken out from time to time, and the concentration

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2) The composition of the isotonic Ringer solution is as follows : 100 parts of M/7.5 NaCl, 2.8 parts of M/7.5 KCl and 3.4 parts of M/11 $CaCl_2$.

of heavy water contained was determined by the pycnometric method. The temperature was regulated by a thermostat at 10.0°±0.5°C. The analytical data obtained are presented in Table 1.

Table 1. Concentration of Heavy Water in the Surrounding Medium.

| Time in hours of exposure | Heavy water in per cent |
|---|---|
| 0 | 1.57 |
| 3 | 1.30 |
| 5 | 0.92 |
| 10 | 0.92 |

It is noted by the tabulated data that the concentration of heavy water contained in the medium decreases with a lapse of time after immersion of the eggs, but observation proved that no further decrease occured later than 5 horus. These data tend to indicate that heavy water permeates into the unactivated eggs from without, and after 5 hours of exposure, the concentration of heavy water within the eggs attains an equilibrium with that of the surrounding medium under a given condition.

According to Krogh and Ussing (1937), the "unfertilized" eggs of the rainbow trout are permeable to heavy water only for the first 6 hours after immersion, but thereafter become impermeable to it. At the end of the experiment, *viz.* 14.6 hours after immersion, an increase of up to 13.8 per cent in the weight of eggs was found. However, on contact with distilled water, or even with fresh water, the unfertilized eggs of the rainbow trout are readily, though abortively activated, like the eggs of the salmon (Kusa, 1950). This activation is invariably accompanied with an inflow of water from without, forming the perivitelline space ; thus, the eggs themselves gain in weight. Such an increase in the weight of the eggs, as obtained by these authors, is nearly equivalent to that found in trout eggs immersed in fresh water, e. g., 11 to 22 per cent in the eggs of *Salmo irideus* and *S. fontinalis* (Bogucki, 1930), and 18 per cent in the eggs of *S. gaidneri* (Manery and Irving, 1935). A comparison of these data suggests that the increase in weight is chiefly due to the uptake of water as a result from onset of activation in the eggs, believed to have taken place in the course of these experiments. It has been show by Krogh himself (1939) that a series of changes occured in the trout eggs on contact with fresh water within one hour, and, in this period, judging from more recent knowledge, the activation of the eggs appears to be completed. Accordingly in Krough and Ussing's experiment, the time of immersion seems to be rather long as compared with the length of time necessary for the completion of the activation in this case. Therefore, in relation to the activation, three different stages of eggs must be superimposed in their experiment : that is, initiation, progression and completion of the activation. As pointed out herein before, unactivated eggs are, to some extent, permeable to heavy water. Hence, concluding that, the unactivated eggs of the trout are naturally endowed with a certain power of permeability to heavy water ; this power

of permeability to heavy water in the eggs is apparently maintained, or even altered over the period of the progress of the activation, but soon abolished after the activation is completed. This is also the case in the eggs completely activated by normal fertilization, as it has been shown also by Krogh and Ussing (1937) that the freshly fertilized eggs of the trout lose this power of permeability. These changes in permeability may be of a greater or lesser degree associated to some changes in the egg metabolism on activation. In some marine invertebrate eggs, such changes as respiratory rate, permeability and differential susceptibility following activation have been generally acknowledged by many authors. In reference to fish eggs, however, no data are yet available except those on the respiratory rate following the fertilization in the eggs of *Fundulus* (Boyd, 1928) and of *Oryzias* (Nakano, unpublished, indirectly cited from Ishida, 1950). The change in the permeability to heavy water is probably also in line with the physiological changes following activation. It would, however, require more experimental data than is now available to prove this.

## Conclusion and Summary

In a heavy-water Ringer, which does not induce the activation, the unfertilized eggs of the rainbow trout, *Salmo irideus*, show to be, in some degree, permeable to heavy water. In the report of an earlier work by Krogh and Ussing (1937), the unfertilized eggs of this species gained considerably weight in dilute heavy water, but it is highly probable that their unfertilized eggs are activated on contact with such salt-free solution as distilled water. Therefore, it may be safe to conclude that a certain degree of permeability to heavy water is endowed naturally with the unactivated eggs of the trout, even though it might be altered by the initiation of activation, and finally abolished, sooner or later, after the activation is completed.

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