

### Mechanism of Action of Extracts of the Posthypophysis on Water Transport through the Skin of the Frog (*Rana esculenta*)

THE increased transport of water through the skin of the frog under the action of posthypophysis extracts<sup>1</sup> has been explained as due to decreased resistance to the water movement<sup>2</sup>. According to this view, an increased absolute diffusion of water in both directions would be expected.

In order to check this hypothesis, isolated frog skin was placed between a known hypotonic or isotonic heavy water (D<sub>2</sub>O) solution (outside) and a Ringer solution of known pH (inside). The molar concentration of heavy water inside was determined after a fixed time interval. In parallel experiments the skin was exposed to posthypophysis extracts (0.5 U./ml.) (Postipofisi, from C. Erba Co., Milan) for the same time, in Ringer solution of about the same pH value. All the experiments were performed at 28 ± 1° C.

For the determination of heavy water we used the technique of Sapirstein<sup>3</sup>; the molar percentage of D<sub>2</sub>O was calculated from the formula:

$$x \text{ per cent} = \frac{1 - \frac{d_0}{cd_1'(1 + 3\beta(t - t_0))}}{\left(\frac{M_2}{M_1} \cdot \frac{d_1}{d_2} - 1\right)d_0 - \left(\frac{M_2}{M_1} - 1\right)} \cdot 100;$$

where  $t_0$  and  $t$  are the temperatures at which the diver floats in ordinary water and in the unknown mixture of ordinary and heavy water respectively,  $d_0$  and  $d_1'$  are the densities of ordinary water at the temperatures  $t_0$  and  $t$  respectively,  $d_2/d_1$  is the ratio of the density of heavy water to that of ordinary water at temperature  $t$ ,  $M_2/M_1$  is the ratio between the molar weights,  $3\beta$  is the thermal coefficient of cubic expansion of 'Pyrex' glass ( $= 10^{-5}$ ) and  $c$  is 0.999984.

From the outside concentrations of heavy water at time 0 and  $t$  ( $C_2^0$  and  $C_2$ ), supposing that the inflow of water is constant and about equal to the outflow, we can calculate the absolute water transfer outside to inside in ml. per hour ( $a_{21}$ ) from the formula:

$$C_2 = \frac{C_2^0}{1 + \frac{V_1^0}{V_2^0}} + \left( C_2^0 - \frac{C_2^0}{1 + \frac{V_1^0}{V_2^0}} \right) \times \exp \left( - \frac{V_1^0 + V_2^0}{V_1^0 \cdot V_2^0} a_{21} t \right),$$

where  $V_1^0$  and  $V_2^0$  are the volumes in ml. of the inside and outside solutions respectively.

Errors due to the difference between volumetric and molar percentages of D<sub>2</sub>O, and to the presence of an equilibrium between D<sub>2</sub>O and DHO, can be neglected for our purposes.

The experimental results are shown in the accompanying table, from which it appears that the absolute transfer of water from outside to inside is 40–80 times greater than the net water transport<sup>1</sup>. The values for the transport of dilute water are in accordance with those of Hevesy *et al.*<sup>4</sup>. Furthermore, the absolute water transfer does not vary with the saline strength of the outside solution, while the net transport decreases with the saline concentration<sup>1</sup>.

Composition of outside solution	Duration of experiment (hr.)	Without posthypophysis		With posthypophysis	
		Inside pH	$a_{21}/\text{cm.}^2$ (ml.)	Inside pH	$a_{21}/\text{cm.}^2$ (ml.)
H <sub>2</sub> O + D <sub>2</sub> O	3-83	7-82	0-215	7-00	0-262
"	1	8-21	0-262	8-15	0-299
"	1	7-50	0-310	7-15	0-310
NaCl 6-5% <sub>00</sub> in H <sub>2</sub> O + D <sub>2</sub> O	1	6-90	0-209	7-15	0-250
"	1	7-30	0-280	7-15	0-280
"	1	7-25	0-305	7-15	0-249

The extracts of posthypophysis do not remarkably alter the absolute transfer of water from outside to inside, although they increase<sup>1</sup> noticeably the rate of net transport of water in the same direction.

It would appear from this work that the posterior lobe principles do not increase the permeability to water in both directions, but that they only increase the inflow of water. This action is presumably only possible with an active mechanism of water transport.

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<sup>1</sup> Capraro, V., and Tiengo, M., *Arch. Sci. Biol.* (in the press).

<sup>2</sup> Sawyer, W. H., *Amer. J. Physiol.*, **164**, 44 (1951).

<sup>3</sup> Sapirstein, L., *J. Lab. Clin. Med.*, **35**, 793 (1950).

<sup>4</sup> Hevesy, G., Hofer, E., and Krogh, A., *Skand. Arch. Physiol.*, **7**, 199 (1935).

### Effect of Diet on the Serum Riboflavin of the Laying Hen

A LARGE number of experiments have been carried out to show that the concentration of riboflavin in the egg of the domestic fowl is directly related to the dietary level<sup>1-3</sup>, and Common *et al.*<sup>4</sup> have shown that a considerable increase occurs in the serum riboflavin of the hen at the onset of laying. However, the relationship of the concentration of riboflavin in the blood serum of the laying hen to riboflavin intake does not appear to have been investigated.

In an investigation of the rate of transfer of riboflavin to the chick embryo during development, hens were fed graded levels of riboflavin in order to produce eggs of varying riboflavin content. At the same time, observations were made on the riboflavin content of the blood serum of the experimental hens. Three groups of hens were kept in specially constructed houses with wire floors and fed on a diet of the following composition: maize meal 45, ground oats 30, wheat bran 10, earthnut cake meal 5, blood meal 4, fish meal 2, limestone 1, steamed bone flour 1, salt 0.5, cod liver oil 1.5, with the addition of folic acid and biotin at 0.25 and 0.15  $\mu\text{gm./gm.}$  diet respectively. Two of the groups received supplementary riboflavin at 2.0 and 4.0  $\mu\text{gm./gm.}$  of diet. Blood samples and eggs were collected at random from each group during the course of the experiment.