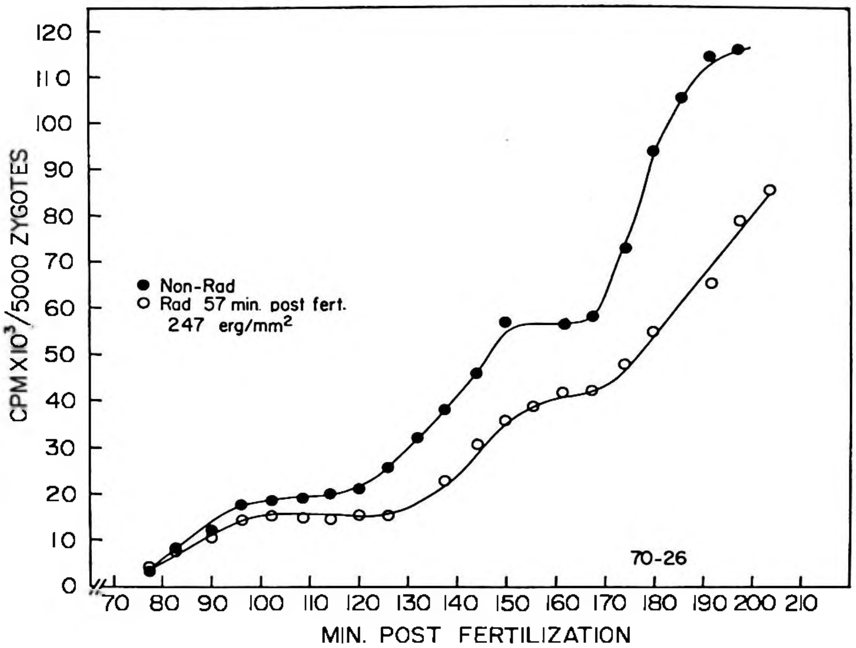

Figure 4.

reactivated indicates that the lesion is most probably a cyclobutane type dimer in the DNA molecule which has been monomerized.

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1970 #36

OSMOTIC AND DIFFUSIONAL WATER PERMEABILITY IN METAMORPHOSING Rana clamitans TADPOLES

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Tadpoles of Rana clamitans metamorphose during the month of July on Mount Desert Island. As the hind legs grow, the tail atrophies. At the stage when the hind legs are about the same length as the tail, the tadpole suddenly changes from gill to lung respiration. Previous experiments on metamorphosing tadpoles showed that the diffusional water permeability is considerably lower in newly metamorphosed frogs than in tadpoles prior to metamorphosis, but insufficient data were obtained to define the change that takes place during metamorphosis (Mackay and Schmidt-Nielsen, Bull. MDIBL 1969). Furthermore, in order to compare the diffusional and the osmotic permeabilities, it was necessary to obtain better data on urine flow.

Rana clamitans tadpoles in various stages of metamorphosis were caught in local ponds during the months of July and August. R. catesbeiana tadpoles were also caught locally, but the data obtained on these, although similar to the data from R. clamitans, are not included here.

The animals were held in the laboratory in running pond water for a week to 10 days prio[r] to the experiments. To determine the rate constant for water turnover, the animals were labeled with tritiated water (THO) by leaving them overnight in labeled water ($5\mu C/ml\ H_2O$). Then each animal was placed in 65 ml of unlabeled pond water which was shaken every minute. Samples of bathing solution (25 μl) were taken every 2 minutes for the first 45 minutes, and every 4 minutes for the last 45 minutes in experiments with tadpoles, and every 3 minutes for the first 45 minutes, and every 4 minutes for the last 2 hours in experiments with frogs. The rate constant "k_{H_2O}" for water turnover was calculated from the exponential decrease betwee[n] the counts in the medium at equilibrium, and the counts in the medium at time t, using the equation,

$$k_{H_2O} = \frac{0.693}{t_{1/2}}.$$

As described earlier (Schmidt-Nielsen and Pagel, 1968), the net diffusional influx ϕ_{net} pe[r] kg of wet body weight can be estimated from the equation:

$$\phi_{net}(ml\ kg^{-1}hr^{-1}) = k_{H_2O}\frac{(hr^{-1})\times \text{body water/body weight}\ (ml\ kg^{-1})\,\Delta Os(M/l)}{55.6\ (M/l)}$$

where ΔOs is the difference in osmotic concentration between the bathing solution and the bod[y] fluids.

As can be seen from the equation, body water and body fluid osmolality must be determin[ed] in addition to the rate constant k_{H_2O}.

Body water was determined by wet weight and dry weight. Plasma osmolality was determined on a Ramsay osmometer on plasma samples from blood obtained by heart puncture.

The urine flow rate was used as a measure of osmotic water influx, since the animal maintained a constant body weight during the experiments.

Urine flow was measured in two ways:

(1) by direct catheterization. A polyethylene catheter (PE 20 to 60) was inserted into the cloaca and held in place by tying it to the tail or body of the animal. The tadpole or frog was then placed in a small plastic sandwich bag with water and a string was tied around the openin[g] of the bag. Urine was collected continuously for 12-24 hours.

(2) by measuring the rate constant for inulin excretion and calculating the filtration rate. The inulin rate constant k_{in} was determined by injecting tadpoles or frogs with 12 μC of ^{14}C inulin, and placing them in 65 ml of H_2O. 25 μl samples were collected and the water changed every 4 hours during the first day, then every 8 hours during the next 2 days. From the following equation:

$$k_{in}(hr^{-1}) = \frac{GFR\ (ml\ kg^{-1}hr^{-1})}{\text{Extracell. fluid vol.}\ (ml\ kg^{-1})}$$

GFR can be calculated if we know the extracellular fluid volume, or the extracellular fluid volume can be calculated if we know GFR.

Direct attempts to measure extracellular volume failed, however, and urine flows were estimated from the equation using measured inulin u/p ratios and an assumed ECF volume of 20 or 30% (Table 2).

The results showed that the decrease in diffusional water permeability takes place rather abruptly during the 2-3 weeks of metamorphosis (Figure 1). It cannot, however, be attributed

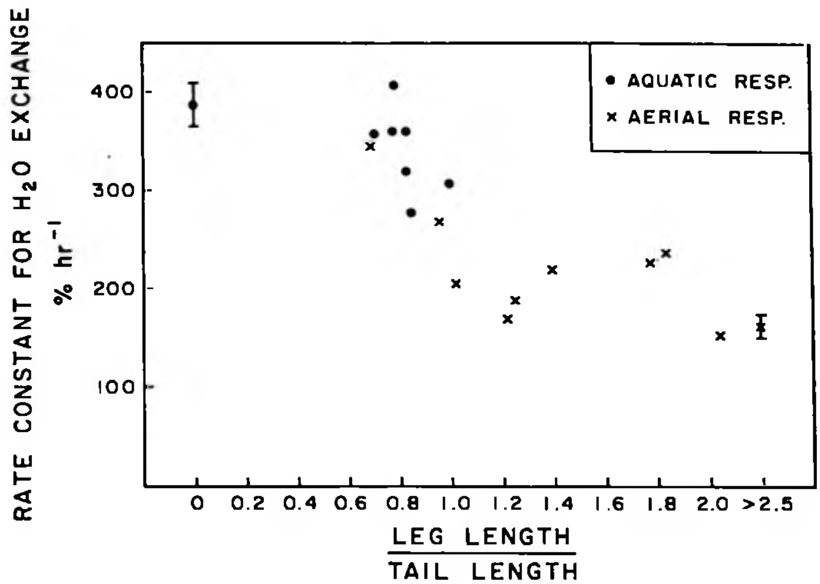

Figure 1. The rate constant for Water (k_{H_2O}) in tadpoles and frogs. Average ± standard error of the mean are given for two series: 9 fully metamorphosed frogs at the extreme right, 12 tadpoles with small hind-legs at the extreme left. The other points represent individual animals.

the change from gill to lung respiration, since the very young, airbreathing frogs showed the same high rate constant as the gillbreathing tadpoles. We must, therefore, assume that the skin permeability changes.

From Table 1, it can be seen that body water content and plasma osmolality are not significantly different before and after metamorphosis. However, the rate constant for inulin excretion is significantly lower in tadpoles than in frogs.

Table 1

	Tadpoles	Frogs
$k_{H_2O}(hr^{-1})$	3.87 ± 0.21 (12)*	1.62 ± 0.11 (9)*
% Body water	87.3 ± 0.5 (15)	82.6 ± 0.9 (12)
Inulin U/P	1.38 ± 0.17 (7)	1.71 ± 0.19 (16)
$k_{in}(hr^{-1})$	0.117 ± 0.010 (13)†	0.259 ± 0.052 (5)†

*$P < 0.001$.
†$P < 0.02$.
Figures in parentheses give numbers of animals used.

In Table 2, calculated and measured urine flows are presented. The measured urine flow in tadpoles is in quite good agreement with the urine flow calculated using the assumption that

Table 2

GFR ml kg^{-1} hr^{-1}	Urine flow ml kg^{-1} hr^{-1}
Tadpoles	
cal. (20% E.F.) 35.1 ± 3.0	25.4 ± 3.8
cal. (30% E.F.) 23.5 ± 2.0	16.9 ± 2.5
measured	21.1 ± 2.1
Frogs	
cal. (20% E.F.) 79 ± 16	46 ± 13
cal. (30% E.F.) 52 ± 10	30 ± 9
measured	23 ± 5

extracellular fluid is 20% of body weight. In frogs this assumption considerably overestimates measured urine flow.

Table 3 gives the calculated osmotic and diffusional water fluxes. It appears that the osmotic and diffusional water fluxes are not significantly different in tadpoles, while in frogs the osmotic flux is about 5 times greater than the diffusional flux. The finding in frogs is in agree-

Table 3

	Tadpoles	Frogs
	ml kg^{-1} hr^{-1}	
Diffusional flux	14.3 ± 0.8	5.72 ± 0.42
Osmotic flux	16.9 ± 2.5	30 ± 9
Osmotic fl./ diffusion/fl.	1.2	5.3

ment with the finding by Hevesy, Hofer and Krogh (Skand. Arch. Physiol. 72:199-214, 1935) an indicates that osmotic flux occurs by bulk flow of water through water filled pores. The findin that the fully aquatic tadpoles do not show significantly different osmotic and diffusional wate fluxes suggests that a change takes place in the structure of the skin between tadpoles and fro

1970 #37

EFFECTS OF BRAIN STIMULATION IN THE HARBOR SEAL (Phoca vitulina)

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We undertook to investigate the possibility of the existence of specific areas in the centr nervous system of the harbor seal (Phoca vitulina) from which circulatory responses resemb the ones obtained during diving, could be elicited by electrical stimulation.