THE NATURE OF WATER TRANSPORT ACROSS FROG SKIN

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ABSTRACT A method has been developed for determining simultaneously short-circuit currents and net water fluxes across frog skin. The basis of the water flux measurement is the determination of changes in weight of a plastic chamber containing the skin and external solution. The accuracy of this method permits net water flows larger than 0.5 mg $cm^{-2}hr.^{-1}$ to be detected, and the apparatus has been used to investigate the relationship between active Na transport and non-osmotic water flow across the skin. Measurement of Na transport and net water influx across completely short-circuited skins provides no good correlation between the two flows. However, skins exhibiting no net water movement in sulfate Ringer displayed an apparent electroosmotic flow of about 40 water molecules per Na ion when depolarizing current densities of 50 and 100 $\mu A\ cm^{-2}$ are used. It is concluded from this and other evidence that the net water influx across frog skin may be partially electroosmotic in character and that there remains another component of water flow unrelated to active Na transport. A theoretical model, based on irreversible thermodynamics, has been developed to explain the non-osmotic water flow across frog skin.

INTRODUCTION

The existence of a net influx of water across isolated frog skin in the absence of osmotic and hydrostatic pressure gradients was first observed unequivocally by Reid (1892). Several workers have claimed that this non-osmotic water flow is coupled to the active sodium transport in the skin but this hypothesis still awaits proof. The inherent difficulties of this problem were previously magnified because no thermodynamic theory of active transport and coupled flows was available, but the present theoretical approach of irreversible thermodynamics may help to settle this outstanding question. Recently Diamond (1962) employed irreversible thermodynamics in a study of water transport across the fish gall bladder *(in vitro)*. Diamond found that the passive diffusion of salt down its activity gradient produced a net flux of water in the same direction across the bladder; such a phenomenon has been termed "codiffusion," and he concluded that the coupling of passive water transport to active solute flow was analogous to this process.

This paper records experiments designed to find a simple description of the nature of non-osmotic water transport across frog skin *in vitro*.

METHODS

The experiments were performed during the winter on the skins of *Rana temporaria* at room temperature (16-20°C). Animals were killed by cutting the spine and pithing; abdominal skin was removed, cleaned of all adherences, and washed in a volume of Ringer or sulfate Ringer. Table I gives the composition of the principal solutions used in this study. All of the salines were buffered with Tris at pH 7.6-7.7 and the use of these media will be indicated in the text or legends to figures by the designated symbols. Salines *A* and *B* will be referred to as Ringer and sulfate Ringer, respectively.

TABLE I

COMPOSITION OF THE EXPERIMENTAL SOLUTIONS

Concentrations are given in millimolar.

	Designation of solution								
	A	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*
NaCl	97.5	–	97.5	97.5	97.5	–	–	–	–
$CaCl_2$	1	–	1	1	1	1	1	1	1
KCl	2.5	–	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Na_2SO_4	–	48.75	–	–	–	–	–	–	–
$CaSO_4$	–	1	–	–	–	–	–	–	–
K_2SO_4	–	1.25	–	–	–	–	–	–	–
Sucrose	–	–	50	100	150	50	150	250	350
Tris	5	5	5	5	5	5	5	5	5

Measurement of Water Flow. A simple gravimetric apparatus capable of measuring short-circuit(*I*) and net water flux (Fig. 1*a*) was constructed from lucite or teflon (polytetrafluorethylene) and net water flow across the skin was determined by changes in chamber-weight after the fluid in compartment *x* had been sucked out through a glass tube (nozzle diameter 1.5 mm) by a vacuum pump. A water trap was included in the suction line and the average fluid evacuation rate was 8 ml/sec.

The procedure for water flux measurements began by filling compartment *y* (volume 1.5 to 5 ml) with solution and placing the skin, corium upwards, over the mouth of *B;* then ring *A* was tightly clamped to chamber *B* by four screws with nuts and washers. Any small pieces of skin protruding between *A* and *B* were removed and the whole chamber was meticulously dried with kleenex tissue. During subsequent handling of the chamber rubber gloves were worn. Volume *x* (1 ml) was filled with solution and this was renewed several times every 15 minutes throughout the entire experiment. After at least 1 hour of equilibration the fluid volume *x* was removed under a binocular dissecting microscope and the chamber was weighed on a Stanton unimatic balance. The fluid volume *x* was replaced, then removed, and the chamber reweighed. After six weighings were completed the weight of the chamber was expressed as a mean ±SD and the chamber was always weighed six times every 2 hours except in one series of experiments (see

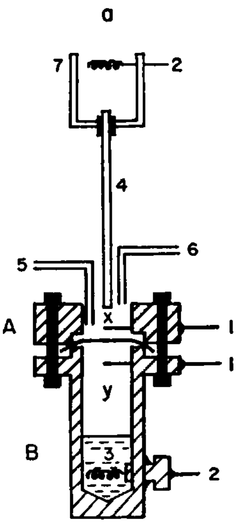

Figure 1*a* Apparatus for measurement of net water flux across frog skin. *A* and *B*, teflon chamber; exposed skin area = 1.25 cm^2. *1*, Ag-AgCl electrodes. *2*, short-circuit current electrodes. *3*, Ringer-agar medium (3 per cent solution). *4*, Ringer-agar bridge. *5*, inlet tube. *6*, outlet tube connected to suction pump. *7*, lucite vessel containing Ringer.

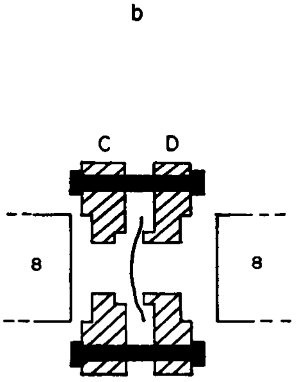

Figure 1*b* Apparatus for measurement of tissue-weight changes. *C* and *D*, lucite rings; exposed skin area = 1.25 cm^2. *8*, lucite chambers analogous to apparatus of Ussing and Zerahn (1951).

Table III) where some observations were performed at hourly intervals. Evaporation from the skin surface produced no significant change in chamber-weight during any single weighing.

The exposed area of skin was 1.25 cm^2 and a typical SD of six weighings was ±0.5 mg. This permitted a net water flux of 0.5 mg $\text{cm}^{-2}\text{hr.}^{-1}$, over a 2-hour period, to be recorded as significant ($P = 0.01$) by Student's *t* test. This value was chosen as the limit of significant measurement of water flow.

Measurement of Potential Difference and Short-Circuit Current. The potential difference was measured with Ag-AgCl electrodes (Fig. 1*a*) prepared immediately before experiments; these electrodes did not differ by more than 1.5 mv during the course of experiments and were connected to a pH-meter No. 22, Radiometer, Copenhagen, by means of small metal plates with holes. Good electrical contact resulted when the connecting wires were under slight mechanical tension. These electrodes were 5 mm apart and no correction was applied for the potential drop in the solution during short-circuiting experiments.

In the lucite chambers the Ag wires were sealed with Araldite; in the teflon chambers the holes for the electrodes were drilled undersize and a good seal obtained by forcing the wires into the chambers. Paraffin wax, applied warm, provided further external sealing when it solidified. The spiral current-electrode in chamber *B* was sealed into a teflon plug allowing easy installation.

Currents, passed across the skin, were measured with a Weston microammeter. The mounting for the internal current-electrode was positioned by a Prior micromanipulator and the fluid volume x was renewed by inlet and outlet glass tubes during continuous short-circuiting of the skin.

Measurement of Tissue Weight Changes. Changes in skin weight in various media were followed in an apparatus (Fig. 1*b*) employing the same drying procedure as in the gravimetric chamber. The skin was clamped between the lucite rings C and D after it had been neatly cut to size (exposed area = 1.25 cm²). This unit was fixed between two lucite chambers similar to the apparatus of Ussing and Zerahn (1951). Changes in tissue weight were determined by weighing the unit CD at 2-hourly intervals as described in the water flux measurements. As there were two surfaces to be dried in any single weighing in these experiments the typical SD of six weighings was larger (±0.8 mg) producing a corresponding decrease in accuracy.

RESULTS

Control Experiments. Control experiments were performed to find if the change of chamber weight could be attributed to the net water flow across the skin only, or if (*a*) leaking, (*b*) water uptake by the chamber material, or (*c*) tissue weight change was the major component. As lucite can take up water this might alter the chamber weight; teflon does not take up water and was a more reliable material on this count.

Paired pieces of skin from the same frogs were set up in the chambers; one skin from each pair served as a control (Ringer both sides) while the other piece was bathed in Ringer containing 1 mM potassium cyanide (KCN). After a 2-hour period of equilibration chamber weight changes were measured over a further 4 hours. In a series of 32 2-hour periods involving 8 pairs, the mean ± SE "net water flux" was: for control skins an "influx" of 1.2 ± 0.3 mg $\text{cm}^{-2}\text{hr.}^{-1}$, and for poisoned skins a "net flux" of 0.0 ± 0.1 mg $\text{cm}^{-2}\text{hr.}^{-1}$. In a similar experiment with 40 2-hour periods involving 10 pairs, in which half were poisoned with 10^{-4} M 2, 4-dinitrophenol (DNP) in Ringer, the mean ± SE net water flux was: for control skins an influx of 1.4 ± 0.2 mg $\text{cm}^{-2}\text{hr.}^{-1}$, and for DNP-treated skins a net flux of 0.0 ± 0.1 mg $\text{cm}^{-2}\text{hr.}^{-1}$. During the control experiments a systematic decrease of the net water influx with time was observed, and this was evident also in later experiments (Table II) despite the maintenance of approximately steady electrical potentials. This observation disagrees with the work of Kirschner *et al.* (1960) who found a tendency for the flow to increase during experiments; these difficulties create the danger of bias in data collected in sequence from skins used in long experiments.

These control experiments strongly suggest that either (*a*) leaking or (*b*) water uptake by chamber material is not a major component of chamber weight change; but it was also essential to know if there were any significant changes in skin weight in the flow experiments. After clamping pieces of skin in the apparatus (Fig. 1*b*) only the internal solution was stirred by aeration to simulate the experimental conditions of the water flow measurements. In a series of 19 2-hour periods involving skins from 11 animals there were only two significant weight changes in skins bathed in Ringer. MacRobbie and Ussing (1961) also found that the "osmotic volume," *i.e.* the stratum germinativum of frog skin, was surprisingly constant.

Tissue weight experiments with poisoned skins were not performed because it seemed highly improbable that absence of net water flux in these skins was caused by fortuitous cancellation of the components of chamber weight change.

Osmosis. MacRobbie and Ussing (1961) obtained estimates of the permeabilities, or hydraulic conductivities, of the two epithelial membranes of frog skin. They found that the outer membrane was relatively more impermeable to water than the inner membrane. It was decided to obtain independently a value for the hydraulic conductivity of the whole skin for comparison. Osmotic experiments were performed on paired pieces of skin from the same animals; one piece from each animal was poisoned by adding 1 mM KCN to all experimental solutions. The skin pairs were subjected to the same conditions of osmotic pressure difference by bathing them with solutions *C, D, E,* and Ringer, and each skin was exposed to one osmotic gradient only. Sucrose was considered to be completely impermeant, and an estimate of the hydraulic conductivity of frog skin was found from a plot of net water flow against osmotic pressure difference across the skin (Fig. 2). In normal and poisoned skins the relationships between net water transport and the net osmotic driving force can be expressed mathematically:

Normal skins $$J_v = (J_v)_o + L_pRT(C_s^i - C_s^o) \tag{1}$$

Poisoned skins $$J_v = L_pRT(C_s^i - C_s^o) \tag{2}$$

where J_v is the net water influx (cm^3 cm^{-2} sec.$^{-1}$); C_s^i and C_s^o the concentrations (mole cm^{-3}) of sucrose in the internal and external media; R, the gas constant; T, the absolute temperature; L_p, the hydraulic conductivity (cm sec.$^{-1}$ atm^{-1}) of frog skin, and $(J_v)_o$ is the rate of fluid transport between identical Ringers in cm^{3}cm^{-2}sec.$^{-1}$.

The simplest description of Fig. 2 is that in normal and poisoned conditions the skin appears to have identical hydraulic conductivities; *i.e.*, $L_p = 3.9 \times 10^{-7}$ cm sec.$^{-1}$ atm^{-1}. This value agrees roughly with MacRobbie and Ussing's estimate of 10^{-7} cm sec.$^{-1}$ atm^{-1} (in sulfate Ringer) for the outer membrane which is the rate-limiting barrier to osmotic water flow. It is also interesting that the non-osmotic water influx, $(J_v)_o$ appears to be unaltered by the different osmotic pressures on the surfaces of the skin.

Recently Kedem and Katchalsky (1963) have shown theoretically that non-linear rate laws may hold for composite membranes (analogous to frog skin) and in view of this and of the effects of sucrose on the electrical resistance of the skin (Ussing and Andersen, 1955), it is surprising that such a high linear correlation

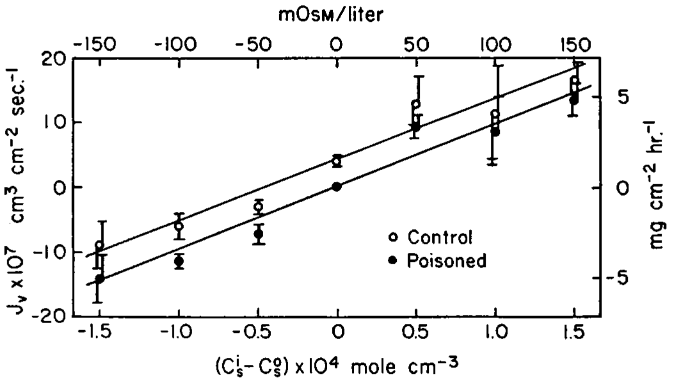

FIGURE 2 Net water flow as a function of osmotic gradient across the skin. The points represent the average values of 10 measurements obtained in 5 experiments using a pair of skins from the same frog for each experiment, one serving as control and one treated with 1 mM KCN. The bars indicate ±SD. Non-zero values of $(C_s^i - C_s^o)$ were produced by bathing one surface of the skin with Ringer and the other surface with *C, D,* or *E.* Regression lines were found for control and poisoned skins:
Control: $J_v = 4.63 \times 10^{-7} + 9.70 \times 10^{-8} (C_s^i - C_s^o)$
Poisoned: $J_v = 9.29 \times 10^{-9} + 9.84 \times 10^{-8} (C_s^i - C_s^o)$
The slope of these lines is given by (L_pRT) and hence L_p was determined.

for osmosis has been found. Coefficients of correlation of the regression lines for control and poisoned skins were $r = 0.9261$ and $r = 0.9425$.

Determination of σ_{NaCl}. Diamond (1962) developed several criteria for deciding whether water transport in the gall bladder was active or passive. His theory rested on three passive properties of the membrane: L_p, the hydraulic conductivity; σ_{NaCl}, the reflection coefficient for NaCl in the membrane and ω_{NaCl} where $(\omega_{NaCl} \cdot RT)$ is the NaCl permeability when there is no volume flow. Staverman (1951) has shown that the osmotic pressure exerted at a membrane by a solution containing a diffusible solute is less than the theoretical value; σ for a given solute and membrane is the ratio of the observed osmotic pressure to the theoretical van't Hoff value.

To measure σ_{NaCl}, a large piece of skin from an animal was cut into four sheets of suitable size for the gravimetric chambers. Each skin had a different external medium (*F, G, H,* or *I*) while the internal medium was Ringer. In this condition there could be no active Na transport and the net water flow across each skin was measured over 4 2-hour periods. Regression lines were fitted to these data and the concentration (C_s^o) of sucrose, when no net water transport occurred, was determined. As sucrose may be considered effectively impermeant, σ_{NaCl} was calculated from $C_s^o = \sigma_{NaCl} C_{NaCl}$, where C_{NaCl} is the osmolarity of NaCl in Ringer. In two experiments regression lines with coefficients of correlation, $r = 0.9720$ and $r = 0.9791$, gave values for C_s^o of 196 ± 26 and 194 ± 22 mOsM/liter (in both cases,

± standard error of estimate). Since $C_{\text{NaCl}} = 181$ mOsM/liter, σ_{NaCl} was found to lie within 0.94 and 1.23 and within 0.95 and 1.19. This parameter was similarly measured for skins poisoned with 1mM KCN and in two experiments regression lines ($r = 0.9670$ and $r = 0.9580$) gave σ_{NaCl} within 0.79 and 1.11 and within 0.90 and 1.25. The precision of these determinations was not high enough to offer a reliable value for σ_{NaCl} other than $0.8 < \sigma_{\text{NaCl}} < 1$.

Relationships between I and Net Water Influx. Capraro and Garampi (1956) suggested that the net water influx across frog skin was electroosmotic in character; *i.e.*, that the net Na transport provided the driving force for the water movement. From data, already obtained independently on net transport of Na and water, the ratio of water molecules (allegedly) dragged per Na ion lies in the range 50 to 300 and it suggested that it might be promising to measure *I* and net water flow across completely short-circuited skins. Fig. 3 shows the results of such experiments on skins from 22 animals. There appears to be no good correlation between Na and water influxes (coefficient of correlation, $r = 0.6651$).

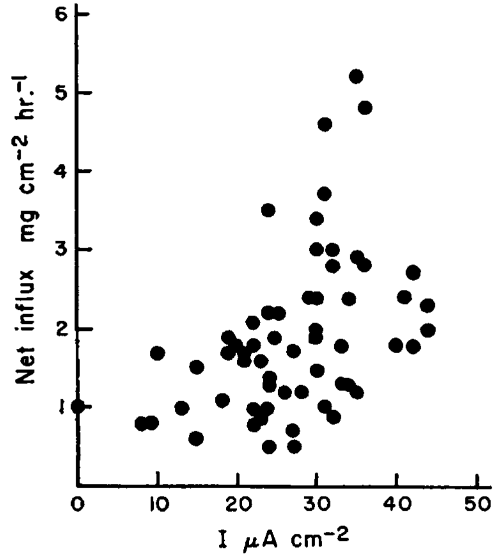

FIGURE 3 Net water influx across completely short-circuited skins as a function of the short-circuit current. As the water flow measurements were taken over 2-hour periods, average values of short-circuit current were calculated (from plots of *I* against time) in the few cases where this parameter was varying significantly.

Fig. 4 shows the effects on *I* and net water influx when the skin is treated with 10^{-5}M ouabain. Ostensibly water influx remains while active Na transport decays, but in four analogous tissue weight experiments with 10^{-5}M ouabain there was an average loss of 5.5 mg during the first 2-hour period while no effective change occurred during the second 2-hour period. These changes of tissue-weight make the interpretation of this experiment complex.

Replacement of External Na by Choline. Kirschner *et al.* (1960) discovered the existence of net water influx (0.8 mg cm^{-2}hr.$^{-1}$) in conditions of no

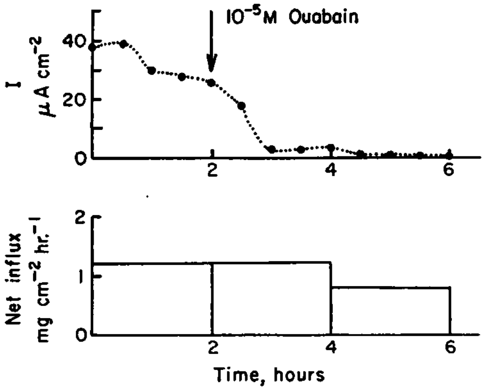

FIGURE 4 The effect of 10^{-5}M ouabain on short-circuit current (I) and net water influx when it is applied to the inside surface of the skin at a time indicated by the arrow.

active Na transport; *i.e.,* external Na was replaced by choline. These results might be explained by a constant tissue swelling and, therefore, the experiments with external choline Ringer were repeated. Choline Ringers were prepared immediately before use and the concentrations of all constituents except sodium were the same; Kirschner *et al.* (1960) have shown that such choline Ringers are isotonic. Table II shows that a net inward movement of water was observed and that this flow was smaller than that of the controls. In Kirschner's experiments a constant entry

TABLE II

WATER INFLUX ACROSS PAIRED FROG SKINS

		Net water influx, *mg* $cm^{-2}hr.^{-1}$		
Animal	External solution	0 to 2 hrs.	2 to 4 hrs.	4 to 6 hrs.
1	Choline Ringer	2.9	1.8	1.4
	Ringer	5.8	3.0	2.0
2	Choline Ringer	0.0	0.0	0.0
	Ringer	1.4	1.3	1.4
3	Choline Ringer	1.9	1.0	0.0
	Ringer	2.0	1.6	0.9
4	Choline Ringer	0.0	0.0	0.0
	Ringer	1.6	1.0	0.8
5	Choline Ringer	0.0	0.0	0.6
	Ringer	1.8	1.9	1.3
6	Choline Ringer	0.8	0.6	0.6
	Ringer	0.0	0.0	0.0
	Choline Ringer	Mean ±SE = 0.6 ± 0.2		
	Ringer	Mean ±SE = 1.5 ± 0.3		

Internal solution in all experiments was Ringer. All net fluxes of water less than 0.5 mg $cm^{-2}hr.^{-1}$ have been called zero.

of water into the skin from the external medium was necessary to explain this phenomenon, whereas in my system that proposal would produce no net change in chamber-weight. It seems safe to consider that this water influx is genuine. However, it may arise from a difference of the osmolalities of the bathing media; this explanation implies an osmotic coefficient for choline chloride of about 0.8 which would have been detected easily in Kirschner's checks (by freezing point depressions) of the total solute concentration in his choline Ringer, and therefore it is considered that this water flux is non-osmotic.

Experiments in Sulfate Ringer. Since the sulfate anion may be considered practically impermeant in frog skin, there can be no net transport of Na ions across skins in sulfate Ringer. Therefore, a study of net water flux across skins in this medium might provide valuable evidence about the linkage, if any, of the two flows. Fig. 5 shows the net water fluxes across 25 skins in sulfate Ringer;

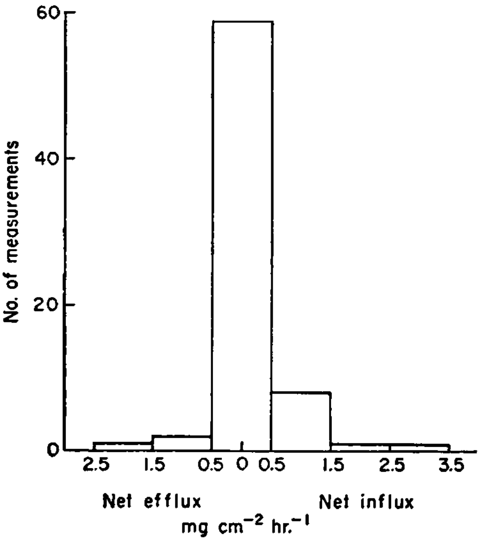

FIGURE 5 Net water movement across skins bathed on both sides by sulfate Ringer.

evidently there is no net water transport when net ionic movement is absent. This situation provides adequate circumstances for testing the effect of passing depolarizing currents across the skin. Table III gives the results of current passage—presumably Na ions—from external to internal medium. These results suggest an electroosmotic drag of about 40 water molecules by every Na ion. An alternative explanation is that the passage of these electrical currents causes a tissue shrinkage; *i.e.*, a loss of water to the internal medium. In five tissue-weight experiments, designed to test this hypothesis, there was no such water loss when currents of 100 μA cm^{-2} were passed over 1-hour periods.

TABLE III

EFFECT OF ELECTRICAL CURRENTS ON WATER TRANSPORT ACROSS FROG SKIN

	Net water influx, $mg\ cm^{-2}hr.^{-1}$		
Skin	0 to 2 hrs.	2 to 4 hrs.*	4 to 6 hrs.
1	0.0	0.0	0.0
2	1.1	1.6	0.8
3	0.0	1.3	0.0
4	0.0	1.8	0.0
5	0.0	2.2	0.0
16	0.0	2.0	0.0
17	0.0	1.0	0.0
18	0.0	1.3	0.0
19	0.0	1.9	0.0
20	0.0	0.6	0.0
Mean ± SE		1.4 ± 0.2	
	0 to 2 hrs.	2 to 3 hrs.‡	3 to 5 hrs.
6	0.0	2.6	0.0
7	0.0	3.5	0.0
8	0.0	2.9	0.0
9	0.0	3.4	0.0
10	0.0	4.1	0.0
11	0.0	2.6	0.0
12	0.6	2.4	0.0
13	0.0	1.9	0.0
14	0.0	2.0	0.0
15	0.0	2.5	0.0
Mean ± SE		2.8 ± 0.2	

Skins were bathed on both sides by sulfate Ringer.

* During this period a current density of 50 $\mu A\ cm^{-2}$ was passed. It is assumed that this current was carried by a net inward movement of Na ions.

‡ A current density of 100 $\mu A\ cm^{-2}$ was passed during this hour.

All net fluxes of water less than 0.5 $mg\ cm^{-2}hr.^{-1}$ in magnitude have been called zero.

DISCUSSION

Codiffusion. Diamond (1962*c*) concluded that net water transport in fish gall bladder was linked to the active NaCl flux by a process analogous to passive codiffusion of salt and water. He calculated expressions for the osmolarity of the absorbate solution on the basis of two different assumptions about the nature of the water movement. Assuming an active water pump and passive solute movement, the osmolarity of the transported solution (equation 7) was:

$$\frac{M_s}{J_v} = \frac{C_s L_p(1 - \sigma_s) - C_s \bar{V}_s \omega_s}{L_p}$$

where M_s is the flux of solute s in moles cm^{-2} sec.$^{-1}$ and $\bar{V}_s$ is the partial molar volume of the solute (milliliters per mole). All other terms have been described before.

The other case considered was the linkage of passive water transport to an active solute pump and the corresponding expression (equation 8) was:

$$\frac{M_s}{J_v} = \frac{\omega_s + C_s L_p(1 - \sigma_s)^2}{L_p(1 - \sigma_s)}$$

The experimental values of these parameters for frog skin are:

$$L_p = 4 \times 10^{-7} \text{ cm sec.}^{-1} \text{ atm}^{-1}$$

$$0.8 < \sigma_{\mathrm{NaCl}} < 1$$

$$\omega_{\mathrm{NaCl}} = 8 \times 10^{-12} \text{ mole cm}^{-2} \text{ sec.}^{-1} \text{ atm}^{-1}$$

$$C_{\mathrm{NaCl}} = 1.8 \times 10^{-4} \text{ mole cm}^{-3}$$

$$\bar{V}_{\mathrm{NaCl}} = 22 \text{ cm}^3 \text{ mole}^{-1}$$

ω_{NaCl} is calculated from (P_{NaCl}/RT) where P_{NaCl} is taken as 2×10^{-7} cm sec.$^{-1}$; this estimate is based on Morel's (1958) value for the Na permeability of the inner membrane. Unfortunately no measurements have been made of P_{NaCl} under conditions of zero volume flow.

Active transport of water: $-0.08 < \frac{M_s}{J_v} < 90$(mOsM | liter)

This solution may be extremely dilute and in the opposite direction to salt movement.

Active transport of solute: $135 < \frac{M_s}{J_v} < \infty$(mOsM | liter)

Since a typical observed value of (M_s/J_v) is (10^{-6} mole cm^{-2} hr.$^{-1}$/2 mg cm^{-2} hr.$^{-1}$) = 10^3 mOsM/liter, Diamond's theory may indicate that water transport is passive and coupled to the Na "pump," but the validity of this is highly dependent on the value for σ_{NaCl}.

In applying Diamond's criteria to frog skin the importance of a precise determination of σ_{NaCl} must be stressed and, in fact, the meaning of this parameter for a composite membrane is problematical since its measurement requires imposing experimental conditions likely to alter the normal passive properties of the membranes in series. Recently Grim (1963) has criticized Diamond's method (used in this study) of measuring σ_{NaCl} because it assumes that water transport is passive; however, Diamond (1962*a*) produced some evidence supporting this assumption. The determination of σ_{NaCl} for frog skin is too inaccurate to decide whether or not metabolic poisoning alters its magnitude.

Electroosmosis. Electroosmosis, which is the flow of water produced by an electric current passing through a charged membrane, has often been invoked to explain anomalous biological water flows; *e.g.,* in the intestine (Parsons and Wingate, 1958) and plant roots (Spanner, 1958). Despite the frequency of this postulation electroosmotic water flow has been observed only once in biological membranes—in a plant cell, *Nitella* (Fensom and Dainty, 1963). These workers observed an electroosmotic transport of water through *Nitella* of about 100 water molecules per ion, and they concluded from the magnitude of the electrical currents driven by "ion pumps" through the cell membranes that the electroosmotic flow was not sufficient to explain the normal high turgor pressure of these cells.

In frog skin the apparent electroosmotic efficiency is not large enough to account for the normal range of non-osmotic water transport; large electrical current densities (40 to 200 μA cm^{-2}) are necessary for the production of water flows in the range (1 to 5 mg cm^{-2} hr.$^{-1}$) normally found. The simplest conclusion is that non-osmotic water transport may be partially electroosmotic in character and that there exists another component of water flow moving independently of Na transport. This latter component is still manifest, for example, when choline Ringer bathes the outside of the skin.

However, electroosmosis can be established only when equality between the streaming potential cross-coefficient and the electroosmotic cross-coefficient is demonstrated. Expressed mathematically this relation is:

$$(\Delta V/\Delta p)_{\Delta\pi, I} = (J_v/I)_{\Delta\pi, \Delta p}$$

where ΔV is the electrical potential difference
Δp the pressure difference in a streaming potential experiment
I is the electric current,
J_v the volume flow in an electroosmosis experiment, and
$\Delta\pi$ the osmotic pressure difference.

The subscripts indicate the flows or forces held at zero.

Streaming potentials have never been observed in frog skin and, moreover, the composite membrane nature of frog skin complicates the definition of proper criteria for the demonstration of streaming potentials and electroosmotic flow. Thus, it cannot be claimed that the existence of electroosmosis in frog skin has been proved unequivocally.

Theory of Non-Osmotic Flow. Experiments reported in this paper support the conclusions of several workers (Kirschner *et al.,* 1960; Capraro and Marro,

1963) that there exists a net inward transport of water across frog skin conditionally unrelated to active transport of Na. Curran and McIntosh (1962) and Ogilvie *et al.* (1963) studied net volume flow across a barrier (analogous to epithelial membranes) composed of two different membranes arranged in series and separated by a closed compartment. They observed a net volume flow across this system against a water activity gradient under certain conditions and suggested that this phenomenon could be explained in terms of the different properties of the membranes and the concentration and pressure gradients across the membranes. A similar theory of non-osmotic water flux across frog skin is offered below.

There are certain features of the model of frog skin, suggested by Koefoed-Johnsen and Ussing (1958), which indicate that there may exist driving forces for the net influx of water. Their model implied a selective permeability of the outer surface of the skin to Na ions while the inner was selective for K ions. Both membranes were permeable to Cl ions. Therefore, it is conceivable that the reflection coefficients of the outer and inner membranes for NaCl and KCl, respectively, may be significantly less than unity.

The following basic assumptions are made:

(1) The ion-pumps maintain a high constant concentration of K ions and a relatively negligible Na concentration between the outer and inner boundaries of the skin.

(2) The ability of the outer membrane to discriminate between KCl and water makes the reflection coefficient for KCl unity.

(3) Similarly, the reflection coefficient of the inner membrane for NaCl is unity.

Consider a composite model of frog skin possessing two membranes in series and suppose that the outer and inner membranes of the skin enclose a fluid having an osmolarity, C_i, of impermeant potassium salt and an osmolarity, C_K, of KCl. Both surfaces of the skin are bathed by identical NaCl Ringer solutions, osmolarity C_N. C_i, C_K, and C_N should be expressed preferably as osmolalities. The volume flows across the two membranes, when no hydrostatic pressure differences exist, are:

$$J_v^o = L_p^o RT[C_K + C_i - \sigma_N^o C_N] \tag{1}$$

$$J_v^i = L_p^i RT[C_N - C_i - \sigma_K^i C_K] \tag{2}$$

where J_v^o = Net volume flow across outer membrane (cm^{3}cm^{-2}sec.$^{-1}$).
J_v^i = Net volume flow across inner membrane.
L_p^o = Hydraulic conductivity of outer membrane (cm sec.$^{-1}$atm^{-1}).
L_p^i = Hydraulic conductivity of inner membrane.
C_K = Osmolarity of KCl in central compartment (moles cm^{-3}).
C_i = Osmolarity of impermeant potassium salt in central compartment.
C_N = Osmolarity of NaCl in external Ringer solutions.
σ_N^o = Reflection coefficient of outer membrane for NaCl.
σ_K^i = Reflection coefficient of inner membrane for KCl.

Equating J_v^o and J_v^i to the net water flux, J_v, across the system, and letting $\lambda = (C_i/C_K)$, then equations (1) and (2) become:

$$L_p^o[C_K(1+\lambda) - \sigma_N^o C_N] = L_p^i[C_N - C_K(\sigma_K^i + \lambda)] = J_v/RT \qquad (3)$$

From equation (3), an equation for C_K follows

$$C_K = \frac{(L_p^o \sigma_N^o + L_p^i)C_N}{L_p^o + L_p^i \sigma_K^i + \lambda(L_p^o + L_p^i)} \qquad (4)$$

Substituting for C_K into equation (3), then

$$J_v = \frac{L_p^o L_p^i RTC_N[1 - \sigma_N^o \sigma_K^i + \lambda(1 - \sigma_N^o)]}{L_p^o(1+\lambda) + L_p^i(\sigma_K^i + \lambda)} \qquad (5)$$

MacRobbie and Ussing (1961) found that (L_p^i/L_p^o) was about 20 and for this model I have taken $L_p^o = 4 \times 10^{-7}$ and $L_p^i = 8 \times 10^{-6}$ cm sec.$^{-1}$ atm^{-1}. From experiments on the diffusion of KCl out of the epithelial cells Ussing (1960) concluded that at least 30 per cent of the osmolarity of the cells was due to KCl. This implies $0 < \lambda < 3$; unfortunately there have been no direct measurements of cellular concentrations of K and Cl ions, although MacRobbie and Ussing estimated that cellular concentration of Cl ions was about 50 mM. Assuming these values for L_p^o and L_p^i, J_v is then plotted against σ_K^i for four values of σ_N^o (Fig. 6) at $\lambda = 3$ and $\lambda = 1$. Both external solutions are taken as Ringer ($C_N = 1.8 \times 10^{-4}$ mole cm^{-3}).

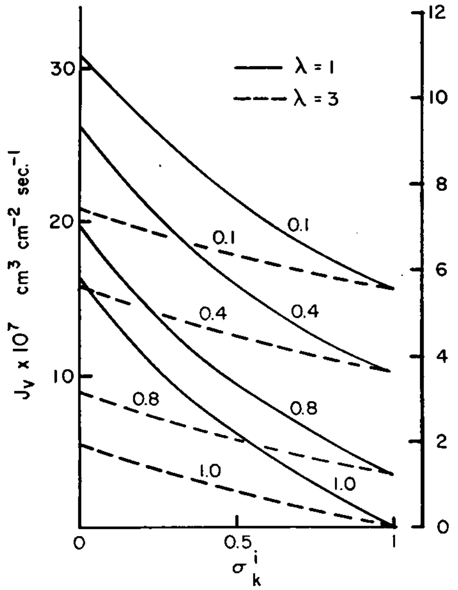

FIGURE 6 The net water influx, J_v, across the membrane system as a function of σ_K^i for $\lambda = 1$ and $\lambda = 3$. The values of σ_N^o (0.1, 0.4, 0.8, and 1) used are shown on the corresponding curves. The ordinate axis on the right of the diagram expresses the predicted J_v in conventional units.

Besides J_v it is also interesting to know what values of C_K are implied by the model. Rearranging equation (4) and substituting $(L_p{}^i/L_p{}^o) = 20$, then

$$C_K = \frac{(\sigma_N{}^o + 20)C_N}{1 + 20\sigma_K{}^i + 21\lambda} \tag{6}$$

Choosing values of $\lambda = 3$ and $\lambda = 1$, C_K is plotted (for extreme values of $\sigma_N{}^o$) against $\sigma_K{}^i$ (Fig. 7). In the extreme case, $\lambda = 0$, (*i.e.* $C_i = 0$) C_K lies in the range 3780 to 186 mOsM/liter.

The theoretical model described above makes three correct predictions about the nature of water transport across frog skin. First, the model quantitatively predicts a net volume flow, J_v, of the experimentally observed magnitude and direction across this system. Secondly, J_v is not directly coupled to active Na transport as it would be if it were completely electroosmotic; however, J_v does depend on metabolically-driven ion-pumps maintaining a constant intracellular medium. Finally, the model predicts the existence of an inward flow of water when inert Ringer (Na replaced by impermeant cation) bathes the external surface; in this case $\sigma_N{}^o = 1$ and J_v is reduced. Moreover, the nature of intracellular medium implied by this

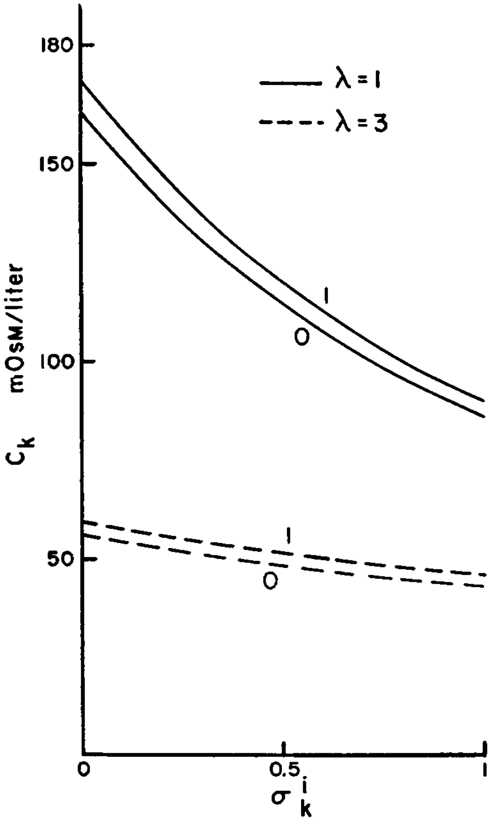

FIGURE 7 The values of C_K, implied by the model, as a function of $\sigma_K{}^i$ for $\lambda = 1$ and $\lambda = 3$. The extreme values of $\sigma_N{}^o$ (0 and 1) used are shown on the corresponding curves.

hypothesis agrees with some experimental facts. Whittembury (1962) found an osmolarity of about 200 mOsM/liter for the osmotically impermeant contents of the cells and, as MacRobbie and Ussing's estimate of the Cl ion concentration was 50mM, it appears that $C_K = 100$ mOsM/liter and $\lambda = 2$ approximately. The model permits approximately these conditions at values of $\sigma_N{}^o$ and $\sigma_K{}^i$ less than unity (Fig. 7).

Until precise determinations of $\sigma_N{}^o$ and $\sigma_K{}^i$ are performed, however, this model can derive support only from its predictions about the behaviour of non-osmotic flow in certain experiments. For instance, an increase in $L_p{}^o$ should produce an increase in J_v and this has been observed experimentally with antidiuretic hormone (C. R. House, unpublished data).

Despite the serious objection that both $\sigma_N{}^o$ and $\sigma_K{}^i$ may equal unity, the model may aid the interpretation of experiments on the net influx of water across frog skin; moreover, this hypothesis provides a simple quantitative description of the water absorption process—the non-osmotic flow of water across frog skin is paradoxically an *osmotic flow*.

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