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# 'UNSTIRRED LAYERS' IN FROG SKIN

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SUMMARY

1. Estimates of the magnitudes of the unstirred regions associated with isolated frog skin in sulphate Ringer's solution have been made under different stirring conditions.

2. The method of investigation was an analysis of the time course of the p.d. transients which occurred when external sodium concentration and internal potassium concentration changes were made in the bathing solution.

3. Making an arbitrary but reasonable assumption about the diffusional coefficient of $Na_2SO_4$ in the outer unstirred region, the magnitudes of the outer unstirred layers were found to lie within the ranges 40–60 $\mu$, 30–50 $\mu$ and 30–40 $\mu$ under stirring conditions of 120, 300 and 500 rev/min, respectively.

4. Making an arbitrary but reasonable assumption about the diffusion coefficient of $K_2SO_4$ in the inner unstirred region, the magnitudes of the inner unstirred layers were found to lie within the ranges 150–230 $\mu$, 120–200 $\mu$ and 100–170 $\mu$ under stirring conditions of 120, 300 and 500 rev/min, respectively.

## INTRODUCTION

In 1936 Teorell wrote that 'ultimately every attempt to study what is hidden in the terms penetration and permeability has to face conditions within diffusion layers', and recently Dainty (1963) and Ginzburg & Katchalsky (1963) have discussed the significance of the 'unstirred layer' in permeability studies on biological and artificial membranes.

The concept of the unstirred layer was originally developed by Noyes & Whitney (1897) and later by Nernst (1904) in their studies of heterogeneous reactions. According to the theory of Nernst, there is a thin layer of static liquid immediately adjacent to the surface of a solid body immersed in fluid. Within this layer, the concentration of solute is a function of position and it is not equal to that in the bulk solution. Experimental measurements have shown that the fluid within such a layer is not

actually stationary; it is a region of slow laminar flow parallel to the solid–liquid interface in which the only mechanism of transport is by diffusion. The thickness of the layer, $\delta$, is an operational quantity defined by

$$\left(\frac{dC}{dx}\right)_{\text{interface}} = \frac{C_b - C_{int}}{\delta}, \tag{1}$$

where $C_b$ is the bulk concentration of the solute and $C_{int}$ is the concentration at the interface. The magnitude of $\delta$ can be *estimated* from hydrodynamic or kinetic measurements and it is usually of the order of $10^{-3}$ to $10^{-2}$ cm. In particular, hydrodynamic studies have demonstrated that $\delta$ is related to the hydrodynamic boundary layer; the latter is defined by the velocity gradient at the solid–liquid interface and is larger than $\delta$.

Nernst assumed that $\delta$ was a constant for a given fluid motion, but current theory (see Levich, 1962) predicts that $\delta$ is a function not only of physical properties and the velocity of the solution, but also of the diffusion coefficient of the solute. This indicates that, under a given regime of motion, the effective thickness of the unstirred layer may not be identical for different substances.

Whether or not these unstirred layers play an important role in membrane transport depends basically on the permeability of the membrane itself to the particular molecular species being transported. Such a layer can be envisaged as an equivalent membrane in series with the actual membrane with a permeability coefficient, $P$, given by

$$P = D/\delta, \tag{2}$$

where $D$ is the diffusion coefficient of the molecular species in aqueous solution. For many solutes $D \simeq 10^{-5}$ cm$^2$ sec$^{-1}$ and the equivalent permeability of an unstirred layer can lie within $10^{-2}$ and $10^{-4}$ cm sec$^{-1}$. These values are close to many quoted permeabilities for rapidly permeating solutes. Hence it is possible that the passage of some solutes across biological membranes may be wholly or partially rate-controlled by diffusion in unstirred regions adjacent to the membrane.

Figure 1 illustrates a possible instantaneous concentration profile during solute permeation across a membrane. $\delta_1$ and $\delta_2$ are the thicknesses of the unstirred layers as defined by eqn. (1).

The application of the Fick equation to such a case, where the flux of solute, $J_s$, is observed, gives

$$J_s = P(\Delta C_s)_{\text{bulk}}, \tag{3}$$

where $P$ is the apparent permeability of the membrane for the particular solute. The 'true' membrane permeability, $P_t$, is given by

$$J_s = P_t(\Delta C_s)_{\text{membrane}}. \tag{4}$$

It can be shown that, under steady-state conditions, $P$ is related to $P_t$ by

$$\frac{1}{P} = \frac{1}{P_t} + \frac{\delta_1}{D_1} + \frac{\delta_2}{D_2} \tag{5}$$

where $D_1$ and $D_2$ are the solute diffusion coefficients in the outer and inner solutions. The existence of these unstirred layers represents a possible serious error in attributing certain properties to the membrane on the basis of $P$, the apparent permeability.

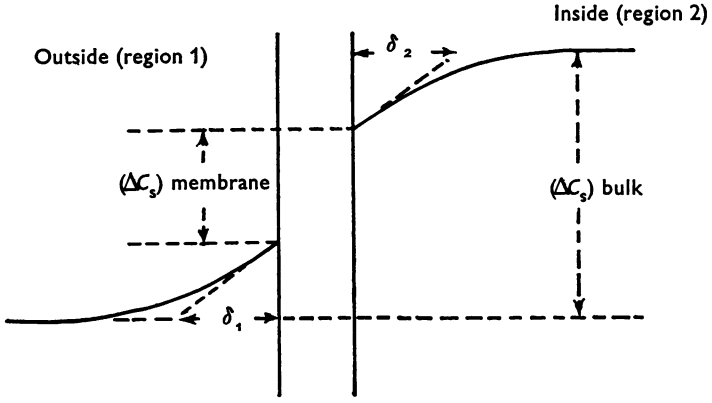


Fig. 1. A possible concentration profile for a permeating solute in the solutions adjacent to the membrane.

A treatment of such a permeation phenomenon in complex biological 'membranes' like epithelia meets the presence of additional intrinsic unstirred regions. In frog skin, for example, such sites of diffusion delay are the corium and the outer epidermis. The present paper is concerned with the estimation of the magnitude of the unstirred regions associated with frog skin *in vitro*.

While this paper was in preparation, Kidder, Cereijido & Curran (1964) published results of a similar investigation on *in vitro* frog skin. These workers performed experiments under moderate stirring conditions only, however, and they were concerned chiefly with a correlation of the magnitudes of the unstirred regions and the localization of the sites of the electrical potential difference within this tissue. Their results are in general agreement with ours.

## METHODS

Experiments were performed during the summer on the skins of *Rana temporaria* at room temperature (17–21° C). Animals were killed by cutting the spine and pithing; abdominal skin was excised, cleaned of adherences and washed in a volume of sulphate Ringer's solution. Table 1 gives the composition of the solutions used; all the salines were buffered with tris at pH 7·6 and the use of these media will be indicated in the text by the designated symbols. Saline *A* will be occasionally referred to as sulphate Ringer.

TABLE 1. Composition of solutions (mM)

| | *A* | *B* | *C* | *D* |
|---|---|---|---|---|
| $Na_2SO_4$ | 48·75 | 37·5 | 25 | 5 |
| $CaSO_4$ | 1 | 1 | 1 | 1 |
| $K_2SO_4$ | 1·25 | 12·5 | 25 | 45 |
| Tris | 5 | 5 | 5 | 5 |

Pieces of skin were mounted between Perspex half-chambers of the type used by Ussing & Zerahn (1951) with an exposed skin area of 1·4 $cm^2$. Approximately 7 ml. of solution bathed each surface, and the potential difference (p.d.) across the skin was monitored through polythene capillaries, filled with 3 % agar in 3 M-KCl, and Pye $Hg/Hg_2Cl_2$/saturated KCl electrodes. The capillaries were of about 1 mm internal diameter. A Pye pH meter was employed as a millivoltmeter and the output was fed into a Honeywell Brown 'Electronik' pen recorder. The recording chart ran at 8 in./min. Bridge asymmetries were checked in sulphate Ringer but no attempt was made to correct for possible junction potentials at the bridges when dissimilar solutions were used.

Vigorous stirring of the bathing solutions was achieved by two stainless-steel bars, within polythene sleeving, which were driven at the same rate by external rotating magnets. The speed of rotation was measured with a stroboscope.

One of the half-chambers was connected through polythene tubing to a suction pump and the solution in this compartment was changed within 2–3 sec by injecting the replacement solution from a large syringe (capacity 50 ml.) through a hole in the top of the half-chamber.

After the skin had been mounted in the apparatus the p.d. was measured frequently until it did not change by more than 1 mV in 30 min. One of the bathing solutions was then changed at a known stirring speed and the p.d. transient was recorded automatically on the moving chart. Estimates of the magnitudes of the unstirred regions on both sides of the skin were obtained from the time course of the p.d. changes; the basis for this quantitative interpretation is now outlined.

## THEORETICAL SECTION

It will be assumed that the p.d. across frog skins in sulphate Ringer is the sum of two diffusion potentials at the outer and inner membranes of the epithelial cells in the stratum germinativum. Koefoed-Johnsen & Ussing (1958) have proposed that in sulphate Ringer the inner membrane behaves like a potassium electrode and the outer as a sodium electrode. For instance, the p.d. across the inner membrane, $\Delta V_i$, is given by

$$\Delta V_i = \alpha \log \frac{[K_c]}{[K_i]} \quad (mV), \tag{6}$$

where $\Delta V_i$ is the potential of the inner solution with the cell interior taken as reference, $[K_c]$ and $[K_i]$ are the concentrations of potassium in the cells and in the inner solution, and $\alpha$ is a constant. In the ideal case, where the inner membrane responds like a potassium electrode, $\alpha = 2{\cdot}3\,RT/F = 58$ mV at room temperature (where $R$ is the gas constant, $T$ the absolute temperature and $F$ the Faraday).

In an experiment where $[K_i]$ is changed from an initial value, $[K_i']$, to a new concentration, $[K_i'']$, it will be assumed that no change occurs in

$[K_c]$ and also that such a concentration change in the bulk solution can be performed instantaneously. For the mathematical treatment of this diffusion problem the frog skin may be represented by an infinite plane sheet. The appropriate equation is

$$\frac{\partial[K_i(x,t)]}{\partial t} = D_K \frac{\partial^2}{\partial x^2}[K_i(x,t)] \tag{7}$$

and the boundary conditions are:

$$t = 0, \quad [K_i] = [K_i'] \quad (0 < x < \delta_i);$$

$$\frac{\partial[K_i]}{\partial x} = 0, \quad x = 0; \quad [K_i] = [K_i''] \quad (x > \delta_i),$$

where $D_K$ is the potassium diffusion coefficient in the region $0 < x < \delta_i$; $x$ is the distance measured from the inner membrane of the epithelial cells in a direction perpendicular to the plane of the skin and towards the inner solution, i.e. $x = 0$ is the interface between the corium and the epithelial cells; and $\delta_i$ is the combined effective thickness of the corium and the unstirred layer in the internal solution.

The solution to a similar diffusion problem can be found in Crank (1957) and, in particular, Olson & Schultz (1942) have calculated values for the concentration at $x = 0$ as a function of time. The half-time, $t_{\frac{1}{2}}$, for the diffusion process is given by

$$t_{\frac{1}{2}} = \frac{0{\cdot}38\delta_i^2}{D_K}, \tag{8}$$

where $t_{\frac{1}{2}}$ is defined as the time taken for $[K_i]$ at $x = 0$ to reach the value $0{\cdot}5$ $([K_i']+[K_i''])$. Equation (8) has been calculated from the data of Olson & Schultz (1942).

A typical p.d. response to such a concentration change is shown in Fig. 2. $\Delta V_0$ and $\Delta V_\infty$ are the initial and final values of the p.d. across the skin; $\Delta V_{\frac{1}{2}}$ is the p.d. after $t_{\frac{1}{2}}$ has elapsed.

It can be shown that

$$\Delta V_\infty - \Delta V_0 = \alpha \log \frac{[K_i']}{[K_i'']}, \tag{9}$$

$$\Delta V_{\frac{1}{2}} - \Delta V_0 = \alpha \log \frac{[K_i']}{0{\cdot}5([K_i']+[K_i''])}. \tag{10}$$

Therefore,

$$\frac{\Delta V_{\frac{1}{2}} - \Delta V_0}{\Delta V_\infty - \Delta V_0} = \frac{\log\{2[K_i']/([K_i']+[K_i''])\}}{\log([K_i']/[K_i''])}. \tag{11}$$

Hence,

$$\Delta V_{\frac{1}{2}} = \Delta V_0 + \frac{(\Delta V_\infty - \Delta V_0)\log\{2[K_i']/([K_i']+[K_i''])\}}{\log([K']/[K_i''])}. \tag{12}$$

Knowing $\Delta V_0$, $\Delta V_\infty$, $[K_i']$ and $[K_i'']$, $\Delta V_{\frac{1}{2}}$ can be calculated; this allows $t_{\frac{1}{2}}$ to be determined from the time record of the p.d. transient and hence $D_K/\delta_i^2$ can be found from equation (8).

The corresponding analysis for the outer membrane yields a similar method of estimating $D_{Na}/\delta_0^2$, where $\delta_0$ is the combined effective thickness of the outer epidermis and the unstirred layer in the external solution, and $D_{Na}$ is the sodium diffusion coefficient in the region $0 < x < \delta_0$. The outer epidermis is composed of two layers called the stratum corneum and stratum granulosum.

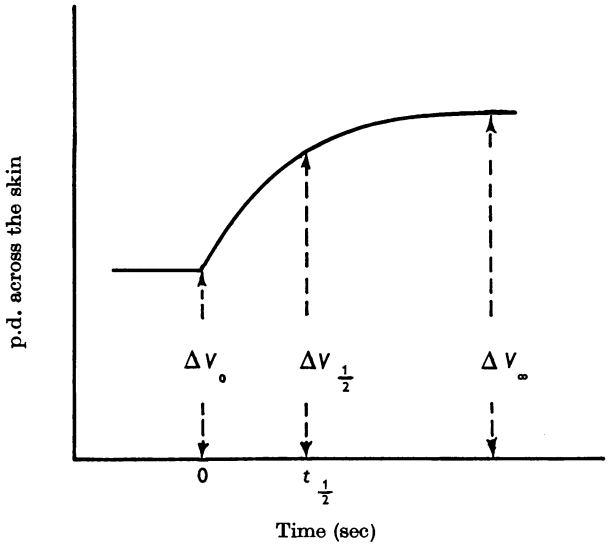


Fig. 2. A typical p.d. response to a change in $[Na_o]$ or $[K_i]$.

In analysing their experimental results Kidder *et al.* (1964) performed a normalizing procedure which reduced all p.d. transient-curves to the same initial and final points but preserved the time course of the change. They defined the fractional change in the p.d. at any time by $F$ where

$$F = \frac{\Delta V - \Delta V_0}{\Delta V_\infty - \Delta V_0}, \tag{13}$$

in which $\Delta V$ is the p.d. at any time after the concentration change. It can be shown that

$$F = \frac{\log([K_i']/[K_{i_0}])}{\log([K_i']/[K_i''])} \tag{14}$$

in which $[K_{i_0}]$ is the potassium concentration at $x = 0$ at any time $t$. The data of Olson & Schultz (1942) permit predictions of $F$ as a function of

time (see eqn. (14)) for given values of $D_K/\delta_i^2$; such theoretical curves can be compared with the experimental values of $F$ obtained from the p.d. transients by eqn. (13).

We have employed this procedure also as a check on our estimates of $D_K/\delta_i^2$ obtained from $t_{\frac{1}{2}}$ values.

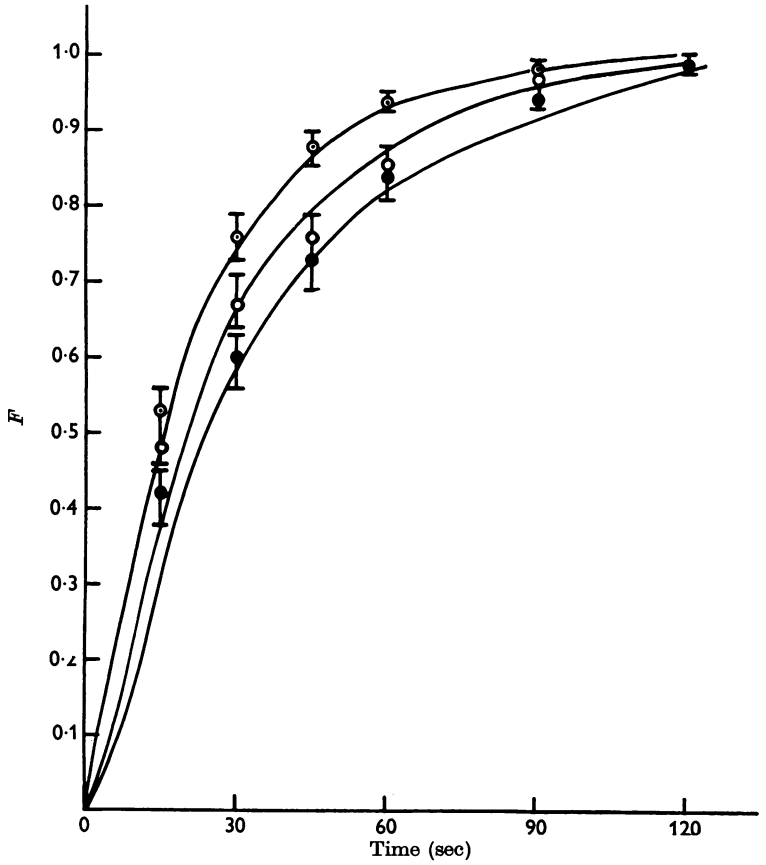


Fig. 3. The fractional change ($F$) in p.d. following a change of the inner solution from $A$ to $B$. Each point is the average of ten measurements on ten skins, ±S.E. ●, ○ and ⊙ denote stirring rates of 120, 300 and 500 rev/min respectively. The lines were calculated from the data of Olson & Schultz (1942) with $D_K/\delta_i^2$ taken as $8{\cdot}4 \times 10^{-3}$, $10{\cdot}4 \times 10^{-3}$ and $13{\cdot}2 \times 10^{-3}$ sec$^{-1}$ for the stirring conditions of 120, 300 and 500 rev/min.

## RESULTS

### *Estimation of* $D_K/\delta_i^2$

The results of a typical experiment, in which the potassium concentration in the internal solution was increased, are shown in Fig. 3. The effect of increasing the stirring rate, for this given change of solution, is to

reduce the value of $t_{\frac{1}{2}}$ obtained from the p.d. transient. Figure 3 shows calculated curves for three values of $D_K/\delta_i^2$ corresponding to the different stirring rates.

Table 2 gives the average results of measurements on twenty animals; these data show an increase in $D_K/\delta_i^2$ as the stirring rate is increased. It is also evident that the values of $D_K/\delta_i^2$ obtained from the transients when $[K_1]$ was increasing are significantly smaller than those found when $[K_1]$ was returning to its value in sulphate Ringer.

TABLE 2. Effect of stirring on estimated values of $D_K/\delta_i^2$

$(D_K/\delta_i^2) \times 10^3$

$(sec^{-1})$

| Stirring rate... (rev/min) | 120 | | 300 | | 500 | |
|---|---|---|---|---|---|---|
| Inner solution | *A* to *B* | *B* to *A* | *A* to *B* | *B* to *A* | *A* to *B* | *B* to *A* |
| Mean ± S.E. | 8·0 ± 0·3 | 19·2 ± 1·0 | 10·4 ± 0·6 | 32·3 ± 1·6 | 13·8 ± 0·7 | 40·0 ± 2·2 |
| Inner solution | *A* to *C* | *C* to *A* | *A* to *C* | *C* to *A* | *A* to *C* | *C* to *A* |
| Mean ± S.E. | 8·3 ± 0·3 | 16·4 ± 1·3 | 9·4 ± 0·3 | 24·4 ± 1·6 | 11·8 ± 0·4 | 32·3 ± 2·2 |

Outer solution in all experiments was sulphate Ringer's solution.

### *Estimation of* $D_{Na}/\delta_o^2$

Table 3 shows the average results of experiments on twenty animals in which the sodium concentration, $[Na_o]$, in the external solution was altered. Again the effect of increasing the vigour of stirring is to decrease the value of $t_{\frac{1}{2}}$.

These data also show similar discrepancies between values of $D_{Na}/\delta_0^2$ at the same stirring rates as were shown in Table 2 for values of $D_K/\delta_i^2$.

TABLE 3. Effect of stirring on estimated values of $D_{Na}/\delta_o^2$

$(D_{Na}/\delta_o^2) \times 10^2$

$(sec^{-1})$

| Stirring rate... (rev/min) | 120 | | 300 | | 500 | |
|---|---|---|---|---|---|---|
| Outer solution | *A* to *C* | *C* to *A* | *A* to *C* | *C* to *A* | *A* to *C* | *C* to *A* |
| Mean ± S.E. | 15·2 ± 0·6 | 10·9 ± 0·3 | 23·7 ± 1·2 | 18·3 ± 1·0 | 26·9 ± 1·3 | 26·9 ± 1·3 |
| Outer solution | *A* to *D* | *D* to *A* | *A* to *D* | *D* to *A* | *A* to *D* | *D* to *A* |
| Mean ± S.E. | 25·3 ± 0·8 | 9·1 ± 0·5 | 38·5 ± 1·5 | 15·2 ± 0·9 | 45·5 ± 1·9 | 21·0 ± 1·5 |

Inner solution in all experiments was sulphate Ringer's solution.

## DISCUSSION

Before one can estimate the magnitude of $\delta_0$ and $\delta_1$ it is necessary to choose reasonable values for $D_{Na}$ and $D_K$. Kidder *et al.* (1964) found that their p.d. transient data, obtained from $[Na_o]$ changes, were best fitted by $20 < \delta_o < 25\,\mu$ and $5 \times 10^{-6} < D_{Na} < 10^{-5}\ cm^2\ sec^{-1}$. In the absence of precise quantitative information their range of values for $D_{Na}$ seems

suitable but these workers were forced to the conclusion that $\delta_0$ was entirely an unstirred layer of solution. They performed similar experiments with a cation exchange membrane and again they obtained a diffusion distance of about 20 $\mu$ under moderate stirring conditions. These results are at variance with those of Ginzburg & Katchalsky (1963) and P. Meares and J. F. Thain (personal communication) who found an unstirred layer of about 25 $\mu$ only under conditions of strong agitation in experiments with artificial membranes. However, there is support for the chosen value for $D_{Na}$ of Kidder *et al.* (1964) in the work of Winn & Fischer (1964) and Winn, Smith, Campbell & Huf (1964) who give $D_{Na}$ (for the corium) = $2{\cdot}6 \times 10^{-6}$ cm$^2$ sec$^{-1}$. Kidder *et al.* (1964) concluded that $0{\cdot}9 \times 10^{-6} < D_K < 2{\cdot}5 \times 10^{-6}$ cm$^2$ sec$^{-1}$ while Hoshiko, Lindley & Edwards (1964) have reported that the $D_{Na}$ (for the corium of bullfrog skin) is about $4{\cdot}2 \times 10^{-6}$ cm$^2$ sec$^{-1}$. On the basis of these measurements we have arbitrarily chosen $D_K = D_{Na} = 4 \times 10^{-6}$ cm$^2$ sec$^{-1}$. Our experiments have shown, therefore, that when the bathing solutions are stirred at 120 rev/min, which is equivalent, perhaps, to the stirring conditions in many previous experiments on frog skin *in vitro*, there exist unstirred regions of about 50 $\mu$ next to the outer membrane and of about 200 $\mu$ at the inner membrane. Unfortunately it is impossible to compare critically these values of $\delta_0$ and $\delta_1$ with the anatomical details of the skin because of the indeterminate nature of the effective thicknesses of the outer epidermis and corium. Under the most vigorous stirring conditions of our experiment (i.e. 500 rev/min) $\delta_0$ and $\delta_1$ fell to about 30 and 140 $\mu$, respectively. The reduction in $\delta_0$ and $\delta_1$ caused by increasing the agitation of the bathing media, however, supports the view that there exist unstirred layers of solution at the skin surfaces; but the unlikely possibility remains that the agitation of the bathing solution may decrease diffusion delay within the tissue itself because of the continuous fluid nature of the system. It is also interesting that a rough hydrodynamical calculation predicts an unstirred layer of about 100 $\mu$ at 120 rev/min; this calculation is based on a solid–liquid interface system.

There are two sources of error in the estimation of $\delta_0$; in these experiments the time course of the p.d. transients was so rapid that the time required for injection of the new solution was about equal to the $t_{\frac{1}{2}}$ value. This invalidates the assumption that concentration changes in the bulk solution could be performed instantaneously and also means that there was an additional mixing effect during the injection of new solution. In these measurements, therefore, 120 rev/min is the lower limit for the stirring rate and the values of $\delta_0$ under these conditions are also lower limits. The second source of error, which also affects the estimation of $\delta_1$, is that there was always some doubt about the exact value of $[K_1]$ or

$[Na_o]$ after a change of solution had been made. This is not considered to be a serious error since even large errors (e.g. $\pm 25\%$) in $[K_i'']$ in eqn. (12) produce small changes in $\Delta V_{\frac{1}{2}}$. Invariably we found that the p.d. changes were satisfactorily reversible and, since these reflect the concentration changes, they support the view that adequate replacement of each solution was achieved by the injection technique.

Unfortunately our analysis has ignored any electrical effects associated with the diffusion of ions in frog skin since we have no knowledge of the fixed charge concentration, if any, within this tissue. The presence of electrically charged sites within the corium, for example, would have a characteristic effect on the kinetics of $[K_1]$ change. Electroneutrality requires that equivalent amounts of counter ion and co-ion be transferred in such a process. Within this possible Donnan system the concentration of the co-ion would be smaller than that of the counter ion; it can be shown that this implies that the electrolyte transfer rate is controlled by diffusion of the co-ion. This rule holds because the electric field has less effect on the ion which is in the minority. Within a fixed-charge system electrolyte transport obeys essentially the rate laws of non-electrolyte diffusion; however, there can be significant deviations when Donnan exclusion is not strong enough to produce a difference between the concentrations of co-ion and counter-ion.

Evidence for the presence of fixed electrical charges within frog skin can be found in the work of Amberson & Klein (1928) and Motokawa (1934*a*, *b*). These investigators found that dead frog skin behaves like an amphoteric membrane which is cation-permeable in solutions on the alkaline side of its iso-electric point, anion-permeable on the acid side and relatively impermeable to highly charged ions of either sign. Dean & Gatty (1937) concluded that the effect of stirring on the p.d. arising from concentration gradients across dead skins (Motokawa, 1934*b*) was probably due to alterations in the ionic diffusion rates. This is consistent with the existence of large unstirred layers in these experiments.

Our treatment of the experimental data has assumed that the diffusion coefficients remain constant and less than those for the diffusion of the salts in free solution. Recently Hoshiko *et al.* (1964) concluded from wash-out tracer experiments on bullfrog skin that the diffusion coefficient of $Na_2SO_4$ within the corium was about one third of its value in free solution. Moreover, their results show the interesting anomaly that the diffusion rate of $Na_2SO_4$ out of the corium is dependent on the composition of the washing medium; for example, the wash-out of this labelled solute is significantly slower when isotonic sucrose bathes the skin. This might indicate the existence of some ion exchange process within the corium.

An interesting discrepancy arising from our results is that the estimated

magnitudes of both $\delta_o$ and $\delta_i$, at each stirring rate were larger in measurements where the values of $[Na_o]$ and $[K_i]$ were being increased. It is possible that this anomaly may reflect some ion exchange properties of the skin, and, indeed, there are several explanations at hand which depend on the differences in mobilities of potassium and sodium ions and also on the operation of possible ionic selectivities. It must be mentioned that Kidder *et al.* (1964) found no such discrepancies.

The importance of our values for $\delta_o$ and $\delta_i$ is that the transport of rapidly permeating solutes may be rate-controlled by these slowly mixing regions rather than by the epithelial cell membranes. Any unstirred layers within cells are likely to be small ($< 20\,\mu$) and, unless the diffusion coefficients are very low within tbe cells, these regions are unlikely to be a source of rate control. Dick (1959, 1964) has drawn attention to the inherent difficulties of studying diffusion within cells, however, and it must be concluded that the diffusion of solutes across an epithelium presents a higher order of complexity. It is particularly relevant that Winn *et al.* (1964) found that nearly all of the resistance to sodium movement in the skin is located in the 'inner epidermis', i.e. stratum germinativum. They also found that, when the epithelial cells became vacuolated as the result of an osmotic treatment, the sodium diffusion coefficient in this layer increased about tenfold. These observations might be considered to indicate the importance of rate-limiting diffusion within the interior of the stratum germinativum; an alternative explanation of these facts involves assumptions about the permeability properties of the epithelial cell membranes. We think that experiments must now be performed to distinguish whether the epithelial cell membrane or the cytoplasm presents the predominant diffusion barrier to solute transfer.

The ability of isolated frog skin, bathed by identical Ringer solutions, to transport salt and water from outside to inside has been confirmed by many investigators. It might be considered that the salt movement provides the driving force for water flow by envisaging that a slowly mixing region like the corium creates a significantly higher salt concentration at the inner boundary of the epithelial cells than that of the bulk solution. In the steady state the next flux of salt, $J_s$, across the region $0 < x < \delta_i$ (and across the skin) will be given by

$$J_w C_s - D_s \frac{dC_s}{dx} = J_s \tag{15}$$

where $J_w$ is the net water influx, $C_s$ is the salt concentration at any point $x$, $D_s$ is the diffusion coefficient of salt within the unstirred regions, and $dC_s/dx$ is the salt concentration gradient at $x$. This equation can be integrated (across the region $0 < x < \delta_i$) to give

$$(C_s)_m = (C_s)_b \exp[-J_w\delta_i/D_s] - (J_s/J_w)\{\exp[-J_w\delta_i/D_s] - 1\}, \tag{16}$$

where $(C_s)^i_m$ is the salt concentration at membrane–corium interface and $(C_s)_b$ is the salt concentration in the inner solution (or in the outer solution) at the unstirred layer boundary. A corresponding calculation for the outer unstirred region gives

$$(C_s)^o_m = (C_s)_b \exp[J_w\delta_o/D_s] + (J_s/J_w)\{1 - \exp[J_w\delta_o/D_s]\}, \tag{17}$$

where $(C_s)^o_m$ is the salt concentration at the outer membrane-unstirred layer interface. If it is assumed that $J_w = L_p RT[(C_s)^i_m - (C_s)^o_m]$, where $L_p$ is the hydraulic conductivity of the skin, then equations (16) and (17) give

$$J_w = \frac{J_s L_p RT(\delta_o + \delta_i)}{D_s + (C_s)_b L_p RT(\delta_o + \delta_i)}. \tag{18}$$

In deriving eqn. 18 we have used the approximation that $\exp(x) = 1 + x$, when $x \ll 1$. Taking $J_s = 10^{-6}$ mole cm$^{-2}$ hr$^{-1}$ (Ussing & Zerahn, 1951), $L_p = 4 \times 10^{-7}$ cm sec$^{-1}$ atm$^{-1}$ (House, 1964), $D_s = 4 \times 10^{-6}$ cm$^2$ sec$^{-1}$ (Hoshiko *et al.* 1964), $C_s = 2 \times 10^{-4}$ mole cm$^{-3}$, $\delta_o = 5 \times 10^{-3}$ and $\delta_i = 2 \times 10^{-2}$ cm, we find that $J_w = 0{\cdot}06$ mg cm$^{-2}$ hr$^{-1}$, which is significantly smaller than the observed net water flux of about 1 to 5 mg cm$^{-2}$ hr$^{-1}$ (Reid, 1892; Kirschner, Maxwell & Fleming, 1960; House, 1964). This mechanism of water transport, therefore, must be discarded.

We have proved, in this paper, that unstirred regions exist on both sides of the effective ionic permeability barriers of the frog skin and, on the basis of reasonable assumptions, have estimated their operational thickness. The salient reason for doing these experiments was to show that the observed values of the permeability of the skin to labelled water required some correction on account of the additional resistances in these unstirred regions. This correction and direct experiments on the effect of stirring on the osmotic and diffusional permeabilities of the skin to water will be described and discussed in a subsequent paper.

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