

THE ENTRANCE OF WATER INTO BEEF AND DOG RED CELLS*

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ABSTRACT

The rate constants for diffusion of THO across the red cell membrane of beef and dog, and the rate of entrance of water into the erythrocytes of these species under an osmotic pressure gradient have been measured. For water entrance into the erythrocyte by diffusion the rate constants are $0.10 \pm 0.02 \text{ msec.}^{-1}$ (beef) and $0.14 \pm 0.03 \text{ msec.}^{-1}$ (dog); the permeability coefficients for water entrance under a pressure gradient of 1 osmol./cm.^3 are $0.28 \frac{\text{cm.}^4}{\text{osm., sec.}}$ (beef) and $0.72 \frac{\text{cm.}^4}{\text{osm., sec.}}$ (dog). These values permit the calculation of an equivalent pore radius for the erythrocyte membrane of 4.1 Å for beef and 7.4 Å for dog. In the beef red cell the change in THO diffusion due to osmotically produced cell volume shifts has been studied. The resistance to THO diffusion increases as the cell volume increases. At the maximum volume, (1.06 times normal), THO diffusion is decreased to 0.84 times the normal rate. This change in diffusion is attributed to swelling of the cellular membrane.

The rate of diffusion of radioactive water (THO) into human red cells has recently been determined (1), as has the rate of water entrance into these cells under an osmotic pressure gradient (2). The results of these two kinds of measurements have been combined to characterize the resistance of the cellular membrane to water entrance in terms of an equivalent pore radius. The present experiments are concerned with an extension of these studies to cells of two other species, beef and dog, using the same techniques for measurement. In addition, diffusion of water into beef cells has been studied after the cells have been allowed to increase or decrease in volume in slightly anisotonic media. When the cells have come to equilibrium with their new environment, the rate of THO exchange with cellular water has again been measured under isosmotic conditions. In this way, small changes in cellular volume have been shown to produce large effects on the diffusion of water into beef red cells.

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Experimental Method and Results

Beef blood was obtained from 21 male animals at a local slaughter house, and used within 6 hours after the animal had been killed. Dog blood was obtained from 16 animals (7 male, 9 female) which weighed between 15 and 20 kilos. No more than 500 ml. of blood was drawn at a single time, and blood was used within 4 hours of being drawn.

TABLE I
Composition of Solutions

Compound	Buffer		Test solutions	
	<i>gm./liter</i>	<i>mM/liter</i>	<i>mM/liter</i>	<i>mM/liter</i>
NaCl.....	6.888	117.8	58.9	235.7
MgCl ₂ ·6H ₂ O.....	0.102	0.5	0.5	0.5
CaCl ₂	0.133	1.2	1.2	1.2
Na ₂ HPO ₄ ·7H ₂ O.....	0.456	1.7	1.7	1.7
NaH ₂ PO ₄ ·H ₂ O.....	0.580	4.2	4.2	4.2
KCl.....	0.330	4.4	4.4	4.4
Na ₂ CO ₃	1.431	13.5	13.5	13.5
Beef				
Osmolarity.....		298	176	546
Total osmolarity after mixing.....		305	216	474
<i>C</i> _{iso} *.....		1.00	0.71	1.55
Dog				
Osmolarity.....		298	176	546
Total osmolarity after mixing.....		304	218	478
<i>C</i> _{iso}		1.00	0.72	1.57

* *C*_{iso} = tonicity in suspension medium employed/isotonic concentration or [*C*_m/*C*₀].

Entrance of Water under Osmotic Pressure Gradient.—

The technique and equipment are the same as those previously described by Sidel and Solomon (2). A suspension of cells was mixed with one of four salt solutions in a mixing chamber. The composition of the salt solutions used is given in Table I. The mixed fluid was forced down a short (and adjustable) length of tube at a velocity of about 200 cm./sec. into a scattering chamber in which the volume of the cells was measured by the intensity of scattered light at an angle of 90° to the incident beam. Observations were made at periods of about 47, 100, 158, and 222 milliseconds after mixing.

The performance of the equipment had been tested previously in experiments on human cells (2). However, since the viscosity of dog and beef blood differs from that of man, a check was made to insure that the flow remained turbulent in the present set of experiments, since turbulent flow is essential for thorough mixing of the moving fluid. In laminar flow, the central fluid moves faster than the peripheral fluid and mixing is poor; under these conditions the flow rate is a linear function of the pres-

sure. Turbulent flow is characterized by a flow rate which is a linear function of the square root of the pressure applied. Such a relationship had previously been found for human red cells; in the present experiments a similar relationship was found with dog and beef blood for flow rates as slow as 151 cm./sec. Since these rates are slower than any used in the course of the present study, it was concluded that the flow was turbulent.

The response of the system to changes in cell volume for beef and dog cells was also checked, and a linear relationship between cell volume and recorded signal was found in each case. Since these control experiments gave results in agreement with those previously obtained for human red cells, it was assumed that the previous controls were also applicable to the present studies in respect to such variables as mixing efficiency and time spent in the mixing chamber.

As discussed by Sidel and Solomon, the time course of cell swelling under an osmotic pressure gradient is given by the following equation (Equations 13 and 14, reference 2):

$$t = \frac{1}{P_s C_{iso}^2} \left[\ln \frac{1 - C_{iso}}{1 - C_{iso}(1 + \Delta V_c / W_{eff} V_{c0})} - \frac{C_{iso} \Delta V_c}{W_{eff} V_{c0}} \right] \quad (1)$$

in which $P_s = (P_w A C_0) / (W_{eff} V_{c0})$ and $C_{iso} = C_m / C_0$. The symbols have the following meaning:

- A = total membrane area
- C_m, C_0 = concentration of solute in the medium at time t , and time zero respectively
- V_c, V_{c0} = volume of the cell at time t , and time zero, respectively.
- $\Delta V_c = V_c - V_{c0}$
- W_{eff} = cellular water apparently free to participate in osmotic phenomena, expressed as a fraction of the cell volume.
- P_w = the cm.³ of water that will cross in 1 second an area of 1 cm.² of cell surface exposed to a concentration difference of 1 osm./cm.³

Experiments in which the entry of water under an osmotic pressure gradient was measured were straightforward except for the determination of W_{eff} , the amount of water which is apparently free to participate in osmotic phenomena. W_{eff} was determined as previously described from the following equation (Equation 15, reference 2):

$$V_c / V_{c0} = 1 + W_{eff}(1/C_{iso} - 1) \quad (2)$$

Fresh, unwashed cells were allowed to equilibrate with suspension mediums of known osmolarity, and the relative cell volume was measured in a Goetz, pear-shaped centrifuge tube, spun at an acceleration of 12,000 g for 45 minutes.

As pointed out by Drabkin (3) and Teorell (4) W_{eff} may consist of two components, one the water content of the cell, and the other an empirical factor which includes contributions due both to the "bound" water of hydration of

the proteins and to the rigidity of the cellular membrane. In the case of the dog, W_{eff} was found to equal 0.70 ± 0.04 , approximately the same as the water content in dog red cells, given by Manery (5) as 72 per cent. This agreement suggests that all the water is free to exchange osmotically, whereas other investigators, quoted by Ponder (6), find about 60 to 75 per cent of the water free to exchange. In view of this discrepancy, the determination of W_{eff} was repeated to make a total of 6 experiments yielding the value given above. In an attempt to determine whether the difference could be ascribed to specific differences in experimental procedure, dog red cells were washed 4 times with isotonic saline, as was done by Parpart and Shull (7). Under these conditions, the discrepancy disappears, and a value of W_{eff} of 0.46 is found which is in good agreement with the value of 0.49 given by Parpart and Shull. Thus W_{eff} appears to depend on the pretreatment of the cell and to reflect strongly the environment of the cellular membrane.

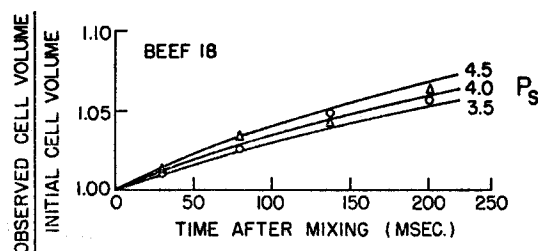


FIG. 1. Fit of the observed experimental data, after conversion to units of relative volume and time, to the theoretical curves. Theoretical curves, according to Equation 1, are shown for P_s values of 3.5, 4.0, and 4.5.

In beef red cells, a value for W_{eff} of 0.35 ± 0.02 has been obtained in a series of 4 experiments. Since the water content of the beef cells is 71 per cent according to MacLeod (8), only 55 per cent of the cellular water is apparently free to exchange, in fair agreement with the values of 57 to 90 per cent as quoted by Ponder.

Equation 1 has been evaluated for all the experimental C_{iso} 's used and for P_s 's from 2 to 7 in steps of 0.5 unit. P_s is then determined graphically, as previously described. Fig. 1 shows the fit of the observed data to the theoretical curve for beef red cells (Experiment B-18). Table II gives the results of the measurements of P_s in the case of the dog and beef. It will be noted that P_s for the dog is 1.5 times that for beef, and hence, that water enters dog red cells under an osmotic pressure gradient faster than it enters beef red cells. Table III gives the results in terms of P_w and other conventional units of measurement.

Diffusion Measurements on Normal Cells.—

The apparatus used has been modified slightly from that previously described by Paganelli and Solomon (1). A suspension of red cells and tritiated isotonic buffer is

TABLE II
Measurement of P_s in Dog and Beef Red Cells

Experiment	Temperature	P_s (sec.) ⁻¹	
Dog			
	°C.	$C_{i80} = 0.72$	1.57
D-10	23	5.0	5.5
D-11	23	5.0	6.0
D-12	23	6.0	6.0
D-13	23	6.0	6.0
D-14	24	6.0	5.5
Average ± s.d.		5.7 ± 0.4	
Beef			
		$C_{i80} = 0.71$	1.55
B-16	22	4.0	3.0
B-17	—	4.0	4.0
B-18	23	4.0	4.0
B-19	23	4.0	4.0
B-20	23	4.0	3.0
B-21	24	4.0	4.0
Average ± s.d.		3.8 ± 0.4	

TABLE III
Water Entrance into Dog and Beef Red Cells under an Osmotic Pressure Gradient

Animal	P_s	P_w	$\frac{P_w}{P_s}$ (per cm. H ₂ O)*	$\frac{P_w}{P_s}$ (per cm. H ₂ O, cell)
	$\frac{1}{\text{sec.}}$	$\frac{\text{cm.}^4}{\text{osm., sec.}}$	$\frac{\text{cm.}}{\text{sec., cm. H}_2\text{O pressure}}$	$\frac{\text{cm.}^3}{\text{sec., cell, cm. H}_2\text{O pressure}}$
Dog †	5.7 ± 0.4	0.72	2.9×10^{-8}	3.6×10^{-14}
Beef ‡	3.8 ± 0.4	0.28	1.1×10^{-8}	1.0×10^{-14}

* The units used are the same as $\text{cm.}^3/(\text{sec., cm.}^2 \text{ area, cm. H}_2\text{O pressure})$.

† Red cell volume = $68 \mu^3$ (9), diameter, 7.1μ (10), area calculated by Emmons' method (11) = $124 \mu^2$.

‡ Red cell volume = $58 \mu^3$ (12), diameter, 5.9μ (10), area calculated by Emmons' method = $92 \mu^2$.

mixed in a chamber and then forced down an observation tube at a velocity of about 9 m./sec. under a pressure of about 2 atmospheres. The pressure forces samples of the suspending medium through ports closed with millipore filters which retain the cells, but allow passage of the suspension medium. In the earlier experiments four ports were spaced along the observation tube so that samples could be taken approximately 2.1, 4.2, 6.2, and 8.3 milliseconds after mixing. In the later experiments, eight ports

were used, allowing observations at about 1.8, 3.8, 5.9, 7.9, 10.0, 12.0, 14.0, and 16.0 milliseconds after mixing. The samples of filtrate are collected from the ports and analyzed for their tritium content as previously described. In view of the control experiments carried out with the cell-swelling device, no separate control experiments were made on the diffusion apparatus.

The rate constant for THO exchange is calculated from the following equation (Equation 8 of reference 1):

$$\frac{p}{p_{\infty}} - 1 = \left(\frac{p_0}{p_{\infty}} - 1 \right) e^{St} \quad (3)$$

in which p = the specific activity of the water of the suspension medium at time, t ; p_0 and p_{∞} refer to initial and infinite time values of p , and $S = -(k/v_q)(p_0/p_{\infty})$. The rate constant, k/v_q , represents the flux of THO per unit volume of cell water, v_q . Some difficulty is introduced when the exchange is characterized by a half-time, rather than a rate constant. Half-times calculated as $-0.693/S$, as done previously (1), are characteristic of each specific experiment. However, since S includes the factor, p_0/p_{∞} , these half-times depend on the hematocrit reading, and will vary from experiment to experiment as the initial conditions change, whereas the rate constant, k/v_q , is independent of initial conditions. Over the time span used in these experiments, the logarithm of the function $\frac{p}{p_{\infty}} - 1$ was found to vary linearly with time. Fig. 2 shows plots of this function against time for representative experiments with both dog and beef red cells. It will be noted that no observation is included at zero time. This value had previously been obtained from a calculation which involved the measured hematocrit reading of the cell suspension before and after mixing. The values at later times, however, are obtained from direct measurement of the THO concentration in the suspension medium. Since the hematocrit reading has no absolute value, but depends upon the acceleration of the centrifuge in which the cells are packed, as well as upon many other variables, values calculated with its use may not be compared on an equal basis with those obtained from direct THO determinations. However, it is necessary to have a value for p_0 in order to obtain k/v_q from the observed slope. For this purpose, p_0 has been obtained from extrapolation of the logarithmic function to zero time.

The results of the experiments on the exchange of water under a diffusion gradient are given in Table IV for dog and beef. The rate constant for THO exchange (k/v_q) in dog red cells is 0.14 ± 0.03 msec.⁻¹, while that in beef red cells is 0.10 ± 0.02 msec.⁻¹.

It is possible to make an approximation of the fraction of the cell volume that is free to exchange with THO. This estimate requires knowledge of the extrapolated zero time THO concentration, the infinite time THO concentration,

and the hematocrit reading, including an estimate of the trapped plasma. Such calculations lead to rough values of 84 per cent for the apparent exchangeable H_2O in the dog red cell, expressed as per cent of cell volume; and 89 per cent for beef red cell. Although there is considerable error in these figures, the values appear to be somewhat higher than those for the water content of the cells which are given as 72 per cent for dog red cells by Manery (5) and 71 per cent for beef red cells by MacLeod (8). Thus it may be possible for other constituents of the red cell to exchange hydrogen for tritium.

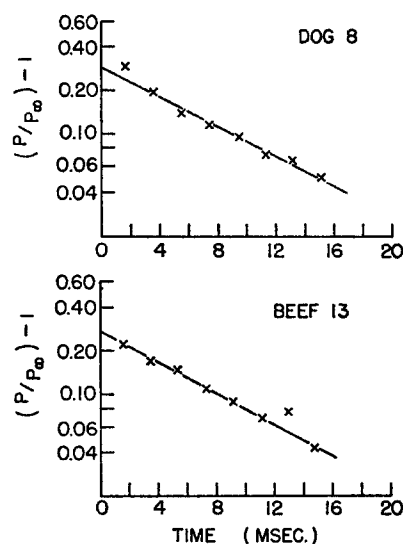


FIG. 2. Time course of THO diffusion into red cells of dog and beef, plotted according to Equation 3.

Diffusion in Beef Cells, as a Function of Cell Volume.—

These experiments were carried out in sets of three so that a single sample of beef blood could be used for simultaneous measurement of diffusion in normal cells, and cells whose volume was slightly larger or smaller than normal. The blood was centrifuged and 40 ml. of plasma was discarded for each 100 ml. of blood. The remainder was divided into three equal samples to which 20 ml. of the appropriate buffer was added for each 100 ml. of initial volume. The osmolarity of the added buffer (Table I, column 2) was adjusted by altering the NaCl concentration to produce final solutions of 0.9 to 1.2 times normal osmolarity. After the cells in the mixture had reached their new equilibrium volume, they were separated by centrifugation. The osmolarity of the supernatant was determined in a freezing point osmometer and tritiated buffers of the same

osmolarity were then immediately prepared. The rest of the experimental procedure was unchanged from that used for normal cells.

Table V shows the results of these experiments over a range of calculated

TABLE IV
*Diffusion Exchange of THO in Red Cells of Dog and Beef**

Animal identification	Hematocrit reading		Flow velocity	k/v_0
	Initial	Final		
Dog				
			<i>cm./sec.</i>	<i>l/msec.</i>
D-1	0.649	0.356	898	0.13
D-2	0.443	0.259	1006	0.11
D-3	0.699	0.365	898	0.13
D-4	0.666	0.333	910	0.16
D-5	0.599	0.314	934	0.19
D-8	0.571	0.310	884	0.092
D-9	0.607	0.308	827	0.13
Average \pm s.d.....				0.14 \pm 0.03
Beef				
B-3	0.495	0.276	928	0.094
B-4	0.517	0.281	910	0.071
B-6	0.591	0.292	935	0.070
B-7	0.512	0.271	910	0.10
B-8	0.592	0.297	915	0.086
B-9	0.565	0.294	941	0.11
B-12	0.689	0.326	902	0.10
B-13	0.574	0.264	896	0.10
B-14	0.567	0.290	853	0.084
B-15	0.557	0.311	881	0.13
Average \pm s.d.....				0.10 \pm 0.02

* 4 observation ports were used in dog experiments, D-1 through D-5, and in beef experiments, B-3 through B-9; eight ports were used in experiments D-8, D-9, and B-12 through B-15.

volume changes from -8.8 to $+6.5$ per cent. The cells from these preparations were examined under a microscope before and after the experiments and were found to retain their biconcave shape.

DISCUSSION

Water Entrance under a Diffusion Gradient.—

From Fig. 2 it can be seen that diffusion of THO into the red cell of the beef is characterized by a single time constant over the time scale used in the present

studies from about 2 msec. after mixing to 16 msec. The final point represents 84 per cent THO exchange. In the case of the dog, diffusion also appears to be linear over the same time scale, and the final point represents 82 per cent THO exchange. Fig. 3 presents the effects of changes in cell volume on the rate of diffusion, as shown in Experiment B-14. We had initially expected that the rate constant for THO entrance would be independent of cellular volume and that the change in slope, S , of the function plotted in Fig. 3 would be less than the fractional change in cell water content. However, the change in slope is much greater than expected. The relationship between the fractional change in the rate constant and the fractional change in cellular volume appears to be linear, as shown in Fig. 4, even though k/v_q at normal cell volume is 0.084/msec. for one beef and 0.134/msec. for the other. It seems improbable that these changes may

TABLE V
Diffusion Exchange of THO in Beef Red Cells under Anisotonic Conditions

Beef identification	C_{iso}^*	Hematocrit reading		Flow velocity <i>cm./sec.</i>	k/v_q <i>msec.⁻¹</i>	Fractional change in k/v_q	Fractional change in cell volume
		Initial	Final				
B-14	1.00	0.567	0.290	853	0.084		
	1.14	0.518	0.264	853	0.111	1.32	0.91
	0.91	0.592	0.296	843	0.076	0.90	1.04
B-15	1.00	0.557	0.311	881	0.134		
	1.19	0.508	0.258	853	0.178	1.33	0.91
	0.93	0.593	0.317	853	0.112	0.84	1.06

* C_{iso} = tonicity in suspension medium employed/isotonic concentration or $[C_m/C_0]$.

be ascribed to gross differences in cell area since Ponder (13) has shown the membrane area to remain essentially constant over volume changes much larger than those employed in these experiments. Furthermore, as previously pointed out, microscopic observation showed that the cells retained their biconcavity in all the suspending mediums employed. It would appear, therefore, that the resistance offered by the membrane to the diffusion of water is a function of the osmolarity of the environment, the resistance increasing as the osmolarity decreases and the cell volume increases. This increased resistance to water diffusion may be ascribed to swelling of the cellular membrane. The membrane would not swell in hypotonic solution, if it were freely permeable to salt, and so it is probable that the barrier for cation entrance lies without the barrier for water diffusion. Such a conclusion is in qualitative agreement with the concept previously put forward (14), of a membrane containing small positively charged pores.

The "permeability" to diffusion for water is denoted by $A_p/\Delta x$, the apparent pore area of the membrane divided by the path length through the membrane

(Equation 9, reference 1). From Ponder's statements it seems reasonable to conclude that the total surface area does not change appreciably under the present conditions, which involve less than a 9 per cent change in cell volume. In consequence, swelling of the membrane might be expected to cause an increase in membrane thickness, which would affect Δx , together with a constriction of the apparent pore area, A_p . In order to obtain a rough estimate of the effect of membrane swelling on membrane dimensions, let us next assume

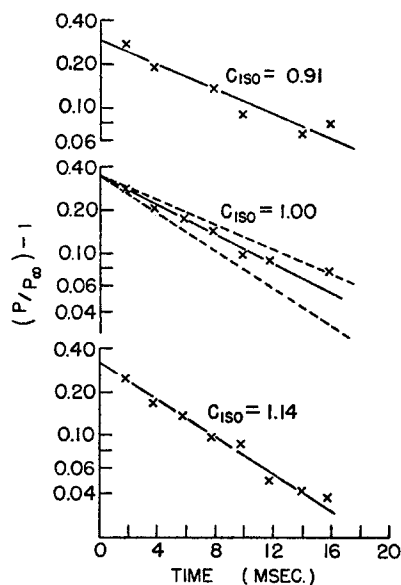


FIG. 3. THO diffusion into beef red cells after small osmotically produced volume changes. The top figure shows diffusion into cells whose volume is 1.04 times normal, and the bottom curve into cells whose volume is 0.91 times normal. The dotted lines in the center section have been obtained by translating the other two curves to the same origin, keeping the slope constant.

that the fractional change in volume of the membrane is the same as the fractional change in total cell volume. The assumption of osmotic behavior of a cellular membrane is in accord with the results of the experiments of Northrop on the swelling of gelatin in water (15). The volume of water taken up by the gelatin depends on the concentration of soluble protein according to the usual osmotic laws, and the swelling of the gelatin is the same in all three dimensions, as long as the elastic limits of the gelatin are not exceeded. A similar conclusion was reached by Northrop and Kunitz (16) for water uptake by gelatin in salt solutions. Thus, the fractional membrane thickness increase would be expected to vary according to the cube root of the fractional change in membrane

volume, and hence the path length through the membrane would probably not increase in a greater proportion. This small increase in path length can surely not account for the increased resistance of the cell membrane to water diffusion, since the slope of the relationship in Fig. 4 shows that the fractional increase in resistance is 3.2 times the fractional increase in cell volume. In view of the difference between $V^{\frac{1}{3}}$ and $3.2V$, this conclusion will not be altered by

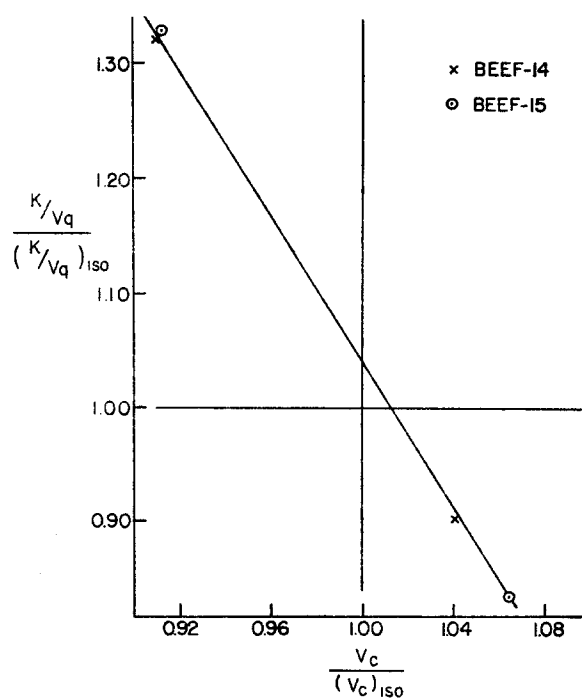


FIG. 4. Fractional change in rate constant for THO entrance into beef red cells as a function of the fractional change in cellular volume.

consideration of differences in the free water content of cell membrane and cell interior.

An extension of the same argument leads to the conclusion that water diffusion into the cells through channels which are large compared to the radius of the water molecule would also not be greatly affected by such small changes in membrane volume. If, however, water diffused into the cells through channels which barely allowed the water to pass, a small change in channel radius should have a very important effect on the rate of diffusion. Such an effect might well be expected in channels of about 5 Å radius, which is consistent with the present model of the beef red cell membrane.

Water Entrance under an Osmotic Pressure Gradient.—

In the diffusion experiments $A_p/\Delta x$ has been shown to be strongly dependent on the osmolarity of the suspension medium; a similar effect on flow under an osmotic pressure gradient should be expected. Osmotic flow under hypotonic conditions, when $C_{\text{iso}} = 0.7$, should be impeded relative to osmotic flow under hypertonic conditions, when $C_{\text{iso}} = 1.6$. However, an examination of Table II reveals that there is no measurable difference between results obtained under these two conditions. Although this observation supports the validity of the osmotic flow measurements, it also raises an important question that is still unresolved. Why is the effect of cell volume change so much greater on diffusion than on osmotic flow? Since osmotic flow depends on the fourth power of the radius of the channel through which water is presumed to enter, and diffusion depends on the square of the radius, the effect should be magnified under an osmotic gradient rather than depressed. The uncertainty in osmotic flow data in the present experiments is of the order of 15 per cent in the constant, P_s , which might mask small changes. After the membrane has reached its equilibrium volume, as is the case for the diffusion experiments, a difference much greater than 15 per cent should be observed. However, the degree of approach to osmotic equilibrium in the osmotic pressure gradient experiments is unknown, since the relative time course of membrane and cell swelling has not been determined.

In the *Arbacia* egg, Lucké, Hartline, and McCutcheon (17) have found a difference in water movement under an osmotic pressure gradient depending upon the osmolarity of the medium. When *Arbacia* eggs are removed from sea water and placed in "0.6 sea water," the rate of water entrance is 80 to 90 per cent as great as the rate of water exit when the converse experiment is carried out and the cells are replaced in sea water. These results are qualitatively similar to those obtained for diffusion in the present experiments on beef red cells. Furthermore, they indicate that entrance under an osmotic pressure gradient is indeed affected by the osmolarity of the suspending medium. However, the time scale for the experiments with *Arbacia* eggs was measured in tens of minutes, which is some 6,000 times greater than the time scale in the present experiments. This may allow membrane swelling to proceed to completion in the *Arbacia* egg. In any case a quantitative comparison is not possible since the *Arbacia* egg possesses a very tight membrane, which has smaller pores than the red cell membrane.

Comparison of Diffusion Permeability with Osmotic Pressure Gradient Permeability.—

In the case of beef, the rate of water entrance from diffusion measurements has been found to be 4.7×10^{-9} cm.³/sec., red cell.¹ The rate of water entrance

¹ This figure has been obtained by multiplying k/v_q by 1.14 to compensate for the difference in diffusion coefficient between THO and H₂O¹⁸, as previously dis-

under an osmotic gradient is 1.0×10^{-14} cm.³/sec., red cell, cm. H₂O pressure. In order to compare these two permeabilities it is necessary to convert the diffusion data into units equivalent to those used for the osmotic data. Since the diffusion rate was obtained from experiments in which the unidirectional flux of THO was measured, the concentration gradient for the diffusion of water was 55.2 mols/liter which is equivalent to 1.38×10^6 cm. H₂O pressure at 25°C. This yields a value of 0.34×10^{-14} cm.³/sec., red cell, cm. H₂O for the diffusion permeability. Thus, the osmotic permeability is 2.9 times greater than the diffusion permeability.

In the case of the dog, the diffusion rate is 7.8×10^{-9} cm.³/sec., red cell, which is equivalent to 0.57×10^{-14} cm.³/sec., red cell, cm. H₂O. The osmotic flow is 3.6×10^{-14} cm.³/sec., red cell, cm. H₂O, 6.3 times greater than the diffusion rate. Such differences in osmotic and diffusion permeabilities are indicative of the presence of water-filled channels in the membrane, as pointed out by Koefoed-Johnsen and Ussing (18).

These experimental data may be used to calculate an equivalent pore radius for the channels in the beef and dog red cells, as was done for the human red cell by Paganelli and Solomon, using the method of Pappenheimer *et al.* (19). The reservations concerning such an interpretation have already been fully discussed (20). Thus, if Poiseuille's law may indeed be applied to pores of such a small size, it is possible to calculate that, on the basis of an idealized membrane containing an array of uniform cylindrical pores, the uncorrected equivalent pore radius is 5.2 Å for the beef and 8.6 Å for the dog.

When the radius of the probing molecule is of the same order of magnitude as the diameter of the equivalent pore, the ratio of the latter should be corrected according to equations given by Renkin (21). These equations lead to corrected equivalent pore radii of 4.1 Å for the beef and 7.4 Å for the dog.

Although no data are available for the approximate membrane thickness of dog and beef red cells the limits of 50 to 5000 Å as used by Paganelli and Solomon for human red cells may be applied to the present studies. The lower limit leads to a value of 0.015 per cent of the total pore area available for diffusion in the case of dog and 0.009 per cent in the case of beef. The upper limits lead to values of 1.5 and 0.9 per cent respectively.

Comparison of Species' Permeability.—

The values of the various permeability coefficients (per unit area of cellular membrane) obtained for beef and dog are compared in Table VI with the values previously given for man. For this purpose a total membrane area of $148 \mu^2$ was calculated for man, using the method of Emmons (11), similar to the calculations in Table III for dog and beef red cell membrane area. The figure

cussed (1). The result, 0.114/msec., is multiplied by the H₂O content of the cell, 4.1×10^{-11} ml., to give the rate of water entrance per cell.

for human red cell area used previously (1) is presumably more accurate than the present figure, but would not be strictly comparable to the others. The question to be examined is whether species' differences in permeability to non-lipide soluble non-electrolytes may be ascribed to differences in the effective pore radius. Differences in the fractional pore area available for diffusion are relatively smaller and may be neglected for the present purpose. From the three pore radii determinations, we would predict a descending order of permeability to non-lipide soluble non-electrolytes as follows: dog > beef > man. However, the order observed by Jacobs (22) is usually: beef > dog > man. For example, the time for hemolysis in distilled water is 1.3 seconds in the beef, 1.8 seconds in the dog, and 2.4 seconds in man (23). This is the same order as that of increasing cell water content in these species, as can be seen in Table VI. A similar order prevails for ethylene glycol and glycerol, after correction for

TABLE VI
Comparison of Species' Permeabilities

Animal	H ₂ O content per cell	H ₂ O entrance		Effective pore radius	Fractional pore area for diffusion	
		By diffusion	Under osmotic pressure gradient		$\Delta x = 50 \text{ \AA} \quad 5000 \text{ \AA}$ per cent	
	ml.	$\frac{\text{cm.}}{\text{sec., cm. H}_2\text{O}}$		\AA		
Beef	4.1×10^{-11}	0.37×10^{-8}	1.1×10^{-8}	4.1	0.009	0.9
Dog	4.9×10^{-11}	0.46×10^{-8}	2.9×10^{-8}	7.4	0.015	1.5
Man	6.3×10^{-11}	0.42×10^{-8}	1.01×10^{-8}	3.5	0.011	1.1

hemolysis time in dilute salt. In the hemolysis method, the measurements involve both filtration and diffusion of the test substance, and the interpretation of the results becomes complex since the cellular volume and the elasticity of the cell membrane both play a part. A formidable number of uncertainties are also involved in the assignment of an equivalent pore radius from the data presently available, so the lack of correlation should not cause undue concern. The ability to predict the relative permeability characteristics of red cells of various species will probably require a more exact knowledge of the architecture of the cellular membrane, and the nature of its chemical constituents.

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