

ENTRANCE OF WATER INTO HUMAN RED CELLS UNDER AN OSMOTIC PRESSURE GRADIENT*

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ABSTRACT

A new technique to determine the rate of water passage through the membrane of the human erythrocyte under an osmotic gradient has been developed. It utilizes a rapid mixing apparatus of the Hartridge-Roughton type which permits measurements at short intervals after the reaction has begun. This is coupled with a light-scattering device of new design which permits the determination of very small changes in volume of the cells without disturbing them. With this technique it was possible to measure the change in volume of freshly drawn human erythrocytes after about 50, 100, 155, and 215 msec. of exposure to anisotonic media. The experimental curves were compared with theoretical curves derived from accepted equations for the process and a permeability coefficient of 0.23 ± 0.03 (cm.⁴/osm., sec.) was obtained.

The present study has been undertaken to measure the rate of water entrance into human red cells under an osmotic pressure gradient. This rate has been measured previously by Jacobs (1) for red cells of the ox and man using the technique of hemolysis time measurement. Recently, however, Jacobs (2) has criticized these earlier measurements on the grounds that delays in rupture of the cell lead to permeability constant values that are too low. The present measurements have been made by a different method, which depends on volume changes smaller than those which produce hemolysis. A modification of the flow method of Hartridge and Roughton (3) permits observation of cell size at intervals of 47, 99, 155, and 216 milliseconds after the cells have been exposed to a variety of anisotonic media; the cell volume in the flowing solution is measured by the intensity of 90° scattered light, a modification of the method used by Ørskov (4) and Parpart (5). Equations developed by Jacobs (6) have been used to determine the rate of water entrance into human red cells from the measured cell swelling curves.

Equipment

Whole blood was mixed with one of four salt solutions in a mixing chamber as shown schematically in Fig. 1. Gas (5 per cent CO₂-95 per cent air) under a pressure of

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about 5 pounds/in.² was used to propel the mixture into the light-scattering chamber at a velocity of about 200 cm./sec. The scattering chamber was illuminated with a beam of white light, and the intensity of the light scattered (and reflected) at an angle of 90° to the incident beam was measured by a photomultiplier. The blood was finally exhausted into a graduate in which the flow rate could be measured with a stop-watch.

All the glassware which came in contact with blood cells was siliconized as routine before use. The 500 ml. bottle containing the blood suspension was placed on a magnetic mixer, and constantly stirred during the experiment. The bottle was carefully shaken by hand immediately before the beginning of each experiment to ensure that the mixture was of uniform composition.

The propelling gas was admitted to the four 100 ml. burettes which contained the salt solutions through glass tubes that extended to the bottom of the burette, thus

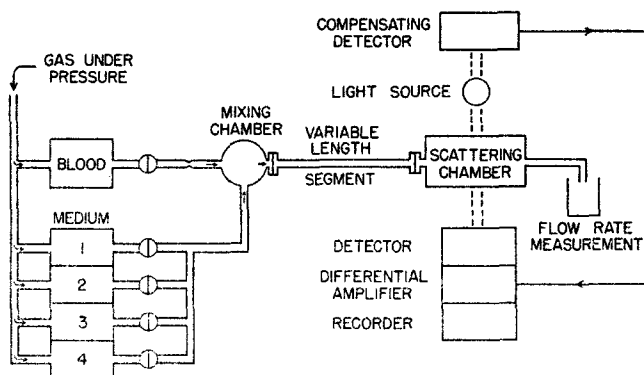


FIG. 1. Schematic diagram of apparatus.

providing a constant flow of fluid independent of the fluid level in the burette, as in Mariotte bottles.

The fluid was led from the various containers to the valve assembly through polyethylene tubing of 2.7 mm. diameter. Stop-cocks could not be used for valves because they could not be opened instantaneously to a reproducible position, and because the grease occasionally occluded the bore causing the rates of flow to differ. To obviate this difficulty, a special valve was constructed using the valve stem from a Hoke No. 456 toggle valve as the actuating mechanism. The details of the assembly are shown in Fig. 2. The four nuts were adjusted to equalize the flow rate for each of the four test solutions. Two valves could easily be opened or closed simultaneously, and the flow through the valve, once adjusted, remained constant for many months. After a great deal of use, the polyethylene tubing hardened, and a different segment was then moved under the valve. An additional clamp was needed on the tubing carrying the blood suspension to throttle the blood flow down to produce a final mixture containing two parts of test solution to each part of blood suspension.

The mixing chamber was constructed after the chamber used by Hartridge and Roughton (their model δ); it is shown in Fig. 2.

Lucite or polyethylene tubing of 0.20 to 0.22 cm. internal diameter connected the

mixing chamber to the scattering chamber. Interchangeable segments were available in lengths ranging from 5 to 50 cm.; these were attached to the chambers with quick change flanges which were clamped together with spring clamps (A. H. Thomas, No. 3241, size 18). Four or five thicknesses of parafilm made a satisfactory gasket.

The scattering chamber is shown schematically in Fig. 3. It was made from a 1 inch right angle prism by the A. D. Jones Co. who bored and polished the 0.218 cm. diameter hole. The hole was countersunk at each end to provide flanges by which it could be clamped to the tubing. On the top a portion of the prism was cut away and

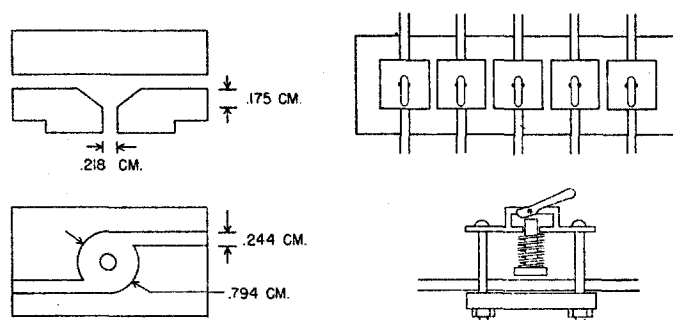


FIG. 2. Left, dimensioned drawing of mixing chamber. Right, side view of single toggle valve, and top view of over-all toggle valve assembly.

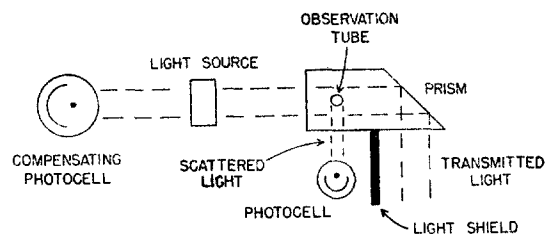


FIG. 3. Schematic drawing showing details of light-scattering chamber and light path.

the surface was roughened to avoid the reflection of scattered light into the optical detector. The truncated prism made an ideal scattering chamber, since the 45° rear surface reflected the transmitted light out of the system.

The light source was a Westinghouse T-8 sound reproducer bulb with a horizontal linear filament (C-6) which was operated from the A.C. supply through a Variac and constant voltage transformer. The light from one side of the bulb passed through a photographic iris and two Wratten A (No. 25) filters before falling on an RCA 931A photomultiplier. In the other direction the light passed through a condenser (American Optical No. 3801-856/520), and three parallel slits before entering the scattering chamber. The detector, positioned at 90° to the incident light, was also an RCA 931A photomultiplier and was well shielded from extraneous light sources.

The photomultipliers were operated at about 700 volts, and the light intensity to

both tubes was matched crudely with the attenuating filter and iris in front of the compensating photomultiplier. The output from the tubes was led into a differential amplifier, from which the difference signal was fed into an Esterline-Angus 1 ma. recorder.

Experimental Method

Blood was drawn from young normal adult human beings into a siliconized flask and mixed with heparin (Lederle, 1000 units/ml.; 5 ml./liter blood). All

TABLE I
Composition of Solutions

Compound	Buffer		Test solutions		
			A	B	C
	gm./liter	mm/liter	mm/liter	mm/liter	mm/liter
NaCl	6.888	117.8	0	58.9	235.7
MgCl ₂ ·6H ₂ O	0.102	0.5	0.5	0.5	0.5
CaCl ₂	0.133	1.2	1.2	1.2	1.2
Na ₂ HPO ₄ ·7H ₂ O	0.456	1.7	1.7	1.7	1.7
NaH ₂ PO ₄ ·H ₂ O	0.580	4.2	4.2	4.2	4.2
KCl	0.330	4.4	4.4	4.4	4.4
Na ₂ CO ₃	1.431	13.5	13.5	13.5	13.5
Osmolarity* (m.osm/liter)		287	72‡	177	545
After mixing 1 part cells in buffer to 2 parts test so- lution, total osmolarity* (m.osm/liter)		290	150‡	220	440
Letting C _{iso} (buffer) = 1, C _{iso} =		1.0	0.52	0.76	1.51

* Measured by freezing point depression (Aminco-Bowman apparatus), using NaCl standards with osmolarity corrected for activity coefficient. The values of 287 m.osm for an isotonic solution given in this line, and 290 m.osm given in the line below, represent two different experimental measurements and agree within experimental error.

‡ Calculated, with corrections for activity coefficients.

blood was used within 6 hours after drawing. Immediately before the start of an experiment, the blood was suspended in normal buffer whose composition is given in Table I (1 part of blood to 2.3 parts of buffer) and aerated with 5 per cent CO₂-95 per cent air, to bring the pH to 7.4. The first burette was filled with normal buffer and the other three burettes with solutions of varying tonicity. These solutions differed from buffer only in the amount of NaCl they contained; their composition is given in Table I. The osmolarity has been normalized to the salt concentration normally found in human blood, so that a value of $C_{iso} = 1.0$ corresponds to a solution containing 287 milliosmols/liter. The values of C_{iso} given in the bottom line of Table I are those which obtain in the solution after it flows out of the mixing chamber.

Experiments were performed by setting the scattering chamber at the shortest distance from the mixing chamber and allowing each of the anisotonic test solutions to mix in turn with the blood suspension. Then the mixing chamber was displaced from the scattering chamber by the introduction of an additional

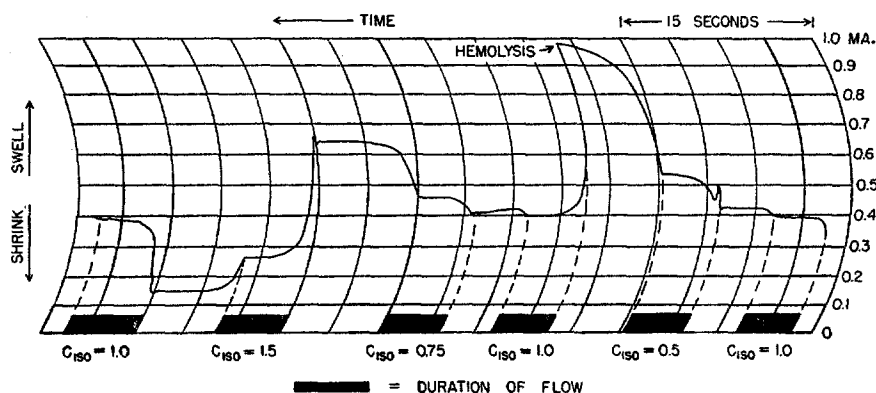


FIG. 4. Recorded performance of light-scattering device during a typical experiment at 155 msec. after mixing. The record runs from right to left. The heavy blocks at the bottom denote periods of solution flow, at the indicated relative concentrations of suspending medium. For the purposes of discussion the record will be divided into six periods, corresponding to the time interval between the initiation of flow of one mixture, and the initiation of flow of the succeeding mixture.

1. ($C_{150} = 1.0$). After initiation of flow, it takes 1 to 2 seconds for the recorder to come to equilibrium. The shift in signal when flow is stopped is constant and characteristic of the system.
2. ($C_{150} = 0.5$). The pulse following the onset of flow is an artifact caused by not turning both valves on simultaneously. When flow is stopped at this concentration, the cells continue to swell until hemolysis occurs, as shown in the figure.
3. ($C_{150} = 1.0$). Return to original baseline indicating constancy of response.
4. ($C_{150} = 0.75$). At this concentration, it will be seen that the cells have swollen to about half the value observed when $C_{150} = 0.5$. When flow is stopped, the cells swell to an equilibrium value short of hemolysis.
5. ($C_{150} = 1.5$). At this concentration, the cells are seen to shrink.
6. ($C_{150} = 1.0$). Return to original baseline.

segment of path length, and the measurements were repeated. Observations were made at four separate times, approximately 47, 99, 155, and 216 msec. after mixing. Normal buffer solution was used as a baseline at the beginning, in the middle, and at the end of the run at each distance. The response of the system to solutions of different tonicities is illustrated in Fig. 4 which shows the response of the system at the 155 msec. period.

RESULTS AND DISCUSSION

*Equipment Performance**Mixing Efficiency.*—

The efficiency of mixing was measured with red cells labelled with Cr^{51} by the method of Gray and Sterling (7). For this purpose, the scattering chamber was replaced with a modified observation tube of the usual diameter. A 26 gauge needle was inserted along the axis of the observation tube so that samples of fluid could be taken near the back wall of the mixing chamber, at the exit from the mixing chamber, and 3 cm. away from the exit. The mixing efficiency was calculated as the ratio of the Cr^{51} radioactivity per unit volume of collected fluid to the Cr^{51} radioactivity per unit volume of effluent fluid after equilibrium had been established. At all the observation points, as shown in Table II, the

TABLE II
Mixing Efficiency

Flow rate	Position of needle	Mixing
<i>cm./sec.</i>		<i>per cent</i>
221	Back wall of chamber	100.1
221	Exit of chamber	97.3
221	3 cm. from exit	100.1
143	Exit of chamber	98.1

number of red cells per unit volume was within 3 per cent of the final equilibrium concentration.

Flow Rate Measurement.—

As the length of segment between the mixing chamber and the scattering chamber increases, the flow rate decreases. Consequently, the flow rate was measured at all four distances used, by timing the flow of a measured volume with a stop-watch. The volume flow rate may then be converted to a linear flow rate by dividing by the cross-sectional area of the tubing. Unfortunately, the internal diameter of the tubing varied from 0.20 cm. to 0.22 cm. The average value has been taken as 0.21 ± 0.01 cm., which leads to a calculated cross-sectional area of 0.034 ± 0.003 cm.². Since the longer lengths were made up by a random choice of shorter segments, the 9 per cent error in tubing cross-sectional area is considered to be a random error.

The reaction time may be obtained from the linear flow rate and the distance from the mixing chamber to the scattering chamber, which includes the equivalent length of the mixing chamber. The latter is determined from the equation given by Hartridge and Roughton (3):

$$L_0 = r_m^2 L_m / r_o^2 \quad (1)$$

in which r_m is the radius, L_m is the length of the mixing chamber, and r_o is the radius of the flow tube. L_o , the equivalent length of the present mixing chamber, is 4.4 cm. This, added to the irreducible minimum distance associated with the scattering chamber, gives 10 cm. as the minimum equivalent length, which represents a time interval of 47 msec., at a pressure of 5 pounds/in.². The added variable segments of 10, 20, or 30 cm. length correspond to increasingly large increments of time due to the slower flow rate at greater lengths. Flow rates varied from a minimum of 185 cm./sec. to a maximum of 214 cm./sec.

The use of Equation 1, which assumes that mixing is complete as soon as the solutions enter the mixing chamber, is probably justified, since Table II shows that mixing is apparently complete at the back wall of the mixing chamber. Furthermore, since the equivalent length of the mixing chamber is only 44 per cent of the minimum distance to the scattering chamber, uncertainties due to the use of Equation 1 are reduced even at the minimum length, and rapidly become unimportant as the length is increased.

The length of the scattering chamber is 2 cm. which represents a possible 20 per cent uncertainty in the time of observation at the closest distance, and a decreasing uncertainty at longer distance. If the entrance of water into the cell is linear with time, the distance to the center of the observation chamber is an accurate measure of the time of reaction, since the variations on either side cancel when the response is integrated over the chamber length. Since, as will be seen from Fig. 8, the observed deviation of the reaction rate from linearity is very small over such a short time interval (approximately 10 msec.), it may be assumed that the time uncertainty arising from the finite length of the observation chamber can be neglected.

Nature of Flow.—

The critical velocity, μ_c , to obtain turbulent flow in the system may be calculated from Reynolds' formula:

$$\mu_c = 1000 \eta / \rho r_o \quad (2)$$

in which η is the viscosity in poises of the suspension, ρ is the density in gm./cm.³, and r_o is the radius of the scattering tube in centimeters. The viscosity of the suspension is obtained from an extrapolation of the data given by Coulter and Pappenheimer (8) for the apparent viscosity (at room temperature) of bovine blood just below turbulence at hematocrit values ranging from 0.18 to 0.53. On the basis of a hematocrit value of 0.05, a viscosity of 0.018 poise, and a density of 1.0, the critical velocity is found to be 165 cm./sec. Since the minimum velocity of flow (obtained at the longest distance in the early experiments) was 185 cm./sec., it seems likely that the flow was turbulent, though the uncertainties in the Reynolds' number for blood suspensions (8) do not lend assurance to this small difference.

In order to examine this point in more detail, an experiment was carried out

in which flow rate was measured as a function of the pressure applied. The results of this experiment, shown in Fig. 5, show that the flow bears a linear relation to the square root of the pressure, down to a flow rate of 162 cm/sec. which is lower than any rates used in the present experiments. Such a result is consonant with turbulent flow, and different from that produced by laminar flow, in which a linear relationship exists between flow and pressure.

Light Scattering as a Measure of Cell Volume.—

A special set of buffers was prepared covering the full range of osmolality used in these experiments. Human red cells were suspended in the buffers at a concentration of one part whole blood in 10 parts total suspension—the same as

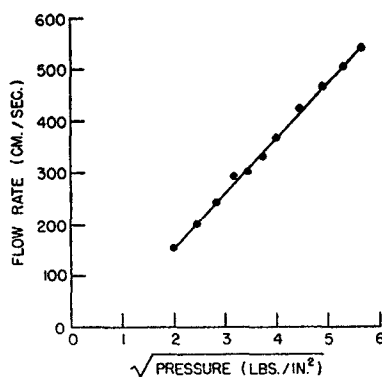


FIG. 5. Flow rate as a function of $(\text{pressure})^{1/2}$. The relationship is linear down to a flow rate of 162 cm./sec., which is lower than any flow rates used in the determination of the permeability coefficient.

used for the final experimental mixture. After the cell volumes in the suspensions had come to equilibrium, the suspension was passed through the scattering chamber at the normal flow rate. An independent measurement of cell volume was made on the effluent fluid using Goetz pear-shaped centrifuge tubes (Kimble 45250), which are well adapted for the measurement of small volumes of cells in the presence of large volumes of buffer. The blood was centrifuged at an acceleration of 1021 *g* for 30 minutes. The calibration of the tubes was checked with weighed volumes of mercury. Fig. 6 shows two calibration curves giving the current in the recorder as a function of cell volume. The difference in the curves reflects a different sensitivity in the two experiments. Since each experiment is calibrated internally, constant sensitivity is not required; it is only necessary that the relationship between cell volume and meter reading be linear in each experiment.

When the flow is stopped during the normal course of an experiment, the cell volumes rapidly come to their equilibrium values, as can be seen from Fig. 4.

The equilibrium levels of the stopped flow mixture for $C_{iso} = 0.75$ and $C_{iso} = 1.5$ have been used to provide the internal calibration. Since the relationship between cell volumes and recorded signal has been shown to be linear over the present range of tonicities, these two points suffice to determine all the cell volumes in a given experiment. The details of the measurement of exact cell volumes for solutions of these tonicities will be discussed below.

Since this method of calibration uses results from stopped flow measurements to calculate volumes obtained during flow, the influence of cell settling on the results was investigated more fully. From Fig. 4, it can be seen that the cells

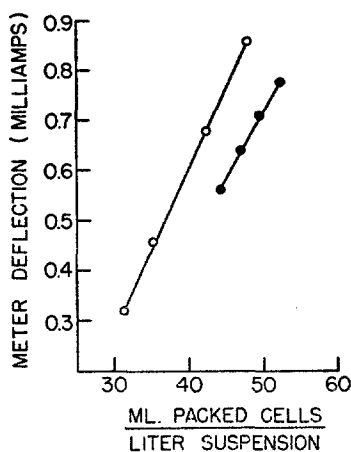


FIG. 6. Calibration curves showing recorder current as a function of cell volume. Each curve represents a single experiment, and the difference between the curves shows a shift in sensitivity between experiments. Since each experiment is calibrated internally, it is not necessary that the sensitivity remain constant.

require less than 5 seconds after flow has stopped to reach their equilibrium volume. Observations for longer times indicate that the effect of cell settling upon the scattered light intensity is a relatively slower process. Typically, the measured signal, under stopped flow conditions, changes uniformly at a rate of about 0.0025 ma./sec. in the direction of cell swelling, independent of the NaCl concentration of the test solution. Since the difference between the volumes for $C_{iso} = 0.75$ and 1.5, rather than the absolute values, is used for the calculation of the cell volumes in an experiment, it appears that the effect of cell settling can be neglected.

Theoretical Considerations

Durbin, Frank, and Solomon (9) have given the following equation to describe net water flow due to the combined effects of bulk flow and diffusion

across an idealized membrane composed of circular pores, of uniform radius, perpendicular to the plane of the membrane:

$$\Delta = \Delta_d + \Delta_f = \phi \Delta \pi (A_w / \Delta x) (D_w \bar{V}_w / RT + r^2 / 8\eta_w) \quad (3)$$

in which

- Δ = net flow in milliliters of water per unit time
- Δ_d, Δ_f = net flow due to diffusion and bulk flow respectively
- ϕ = practical osmotic coefficient
- $\Delta \pi$ = $RT\Delta C$, the van't Hoff equation (R the gas constant; T the absolute temperature; ΔC the concentration difference, expressed in osmolar units)
- A_w = total pore area for water (cm^2)
- Δx = pore length (cm.)
- r = pore radius (cm.)
- D_w, \bar{V}_w, η_w = respectively, diffusion coefficient, partial molal volume, and viscosity of water.

If we assume that conditions are ideal, $\phi = 1$, and the van't Hoff relation holds,

$$\Delta = \Delta C (A_w / \Delta x) (D_w \bar{V}_w + r^2 RT / 8\eta_w) \quad (4)$$

In the case of the human red cell, placed in a solution containing non-penetrating solutes only,

$$\begin{aligned} \Delta &= dV_w / dt \\ \Delta C &= C - C_m \end{aligned} \quad (5)$$

in which V_w is the volume of cell water, expressed in milliliters and C and C_m are the concentration of solutes within the cell, and the medium respectively, expressed in osmols/ml. As water enters the cell, C will vary as follows:

$$C = C_o V_{wo} / V_w \quad (6)$$

in which the subscript o refers to initial conditions. Equation 4 then becomes

$$dV_w / dt = (A_w / \Delta x) (D_w \bar{V}_w + r^2 RT / 8\eta_w) (C_o V_{wo} / V_w - C_m) \quad (7)$$

Equation 7 is equivalent to that given by Jacobs (Reference 6, Equation 2) to describe the entrance of water into cells in the absence of penetrating solute. Jacobs' equation, with a slight change of symbols, is

$$(dV_w / dt) = P_w A (C_o V_{wo} / V_w - C_m) \quad (8)$$

from which it can be seen that

$$P_w = [(D_w \bar{V}_w + r^2 RT / 8\eta_w) / \Delta x] (A_w / A) \quad [\text{cm}^4 / \text{osm.}, \text{sec.}] \quad (9)$$

Since A_w is not, in general, known, Jacobs has expressed the area in terms of A , the total membrane area, and the last factor on the right of Equation 9 has been

introduced to conform with this usage. As Jacobs (1) has pointed out, the area of the cellular membrane remains constant over wide changes in cell volume. Treating A as a constant, Equation 8 may be integrated to give

$$t = \frac{1}{P_w A C_m^2} \left[C_o V_{wo} \ln \frac{C_o V_{wo} - C_m V_{wo}}{C_o V_{wo} - C_m V_w} - C_m (V_w - V_{wo}) \right] \quad (10)$$

Since it is more convenient to express concentrations in units of C_{iso} , the isotonic concentration, and volume in terms of V_c , the cell volume, the following transformations are carried out:

$$C_{iso} = C_m / C_o \quad (11)$$

$$V_c = V_{eo}(1 - W_{eff}) + V_w \quad (12)$$

W_{eff} is the cellular water which apparently participates in osmotic phenomena, expressed as a fraction of the cell volume. Setting $\Delta V_c = V_c - V_{eo}$. Equation 9 becomes,

$$t = \frac{W_{eff} V_{eo}}{P_w A C_{iso}^2 C_o} \left[\ln \frac{1 - C_{iso}}{1 - C_{iso}(1 + \Delta V_c / W_{eff} V_{eo})} - \frac{C_{iso} \Delta V_c}{W_{eff} V_{eo}} \right] \quad (13)$$

For convenience, set

$$P_t = \frac{P_w A C_o}{W_{eff} V_{eo}} \quad (\text{sec.})^{-1} \quad (14)$$

Equation 13 has been evaluated for the three experimental C_{iso} 's used (0.5, 0.75, 1.5) and a range of P_t 's from 1.0 to 4.0.

There is a great body of experimental evidence, summarized by Ponder (10), which suggests that the human red cell acts osmotically as if an appreciable fraction of the cell water were not free to participate in osmotic phenomena. Consequently experiments have been carried out to relate the volume of cells at equilibrium to the osmolality of the suspending medium, and thus to determine W_{eff} , the osmotically active cell water, under the present experimental conditions. Ponder's equation relating these variables in dilute suspensions is equivalent to the one given below

$$V_c / V_{eo} = 1 + W_{eff}(1/C_{iso} - 1) \quad (15)$$

The results of one such experiment are presented in Fig. 7, in which measured cell volume is plotted against $(1/C_{iso} - 1)$. The cell volumes were measured using the special hematocrit tubes and centrifuge described by Gold and Solomon (11). The slope of the curve gives a value of 0.46 for W_{eff} . Since Hald *et al.* (12) give a value of 722 gm./liter red cells for the water content of the human red cell, only 64 per cent of the cell water appears to be free to participate in osmotic phenomena. This figure, though a little low, lies in the normal range of previous data, as summarized by Ponder (10). The reason for this pecu-

liar behavior is still not clear, and as Ponder (13) describes in a recent review, arguments have been advanced to account for it both on the basis of "bound" water (water of hydration of the proteins), and rigidity of the cellular membrane.

Measurement of Permeability Coefficient

The time course of the volume change in a typical experiment is shown in Fig. 8. The figures for signal change in milliamperes are obtained from the arithmetical difference between the signal obtained with the test solution and that obtained with the isotonic buffer, which served as the baseline for each distance. As can be seen from Fig. 4, the baseline is essentially constant through-

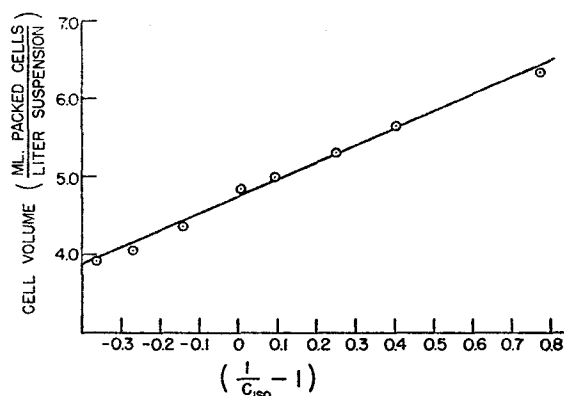


FIG. 7. Cell volume as a function of the salt concentration in the suspending medium.

out a run at any given distance; as the distance is changed, it varies less than 0.01 ma.

It will be noted that none of the extrapolated curves goes through zero at zero distance. Shifts such as those shown in Fig. 8 represent a constant and characteristic feature of the experiments. Indeed the crossover point of the three curves is relatively constant, occurring usually at a time of 30 to 40 msec. after mixing. The shift cannot, however, be ascribed to a delay in mixing, since the experimental results given in Table II show that mixing is almost instantaneously complete. Although the ionic environment in which the cells find themselves at the instant of mixing varies only with respect to NaCl concentration (and pH), all other constituents being kept constant, the changes both in NaCl concentration and in ionic strength are appreciable. We have tentatively, therefore, ascribed the zero time shift to the changes in NaCl concentration, but have no suggestions to put forward as to the detailed mechanism responsible.

It is necessary to convert to units of time and isovolume (the ratio of observed

cell volume to the initial cell volume) in order to obtain permeability coefficients from data similar to those given in Fig. 8. Since the response of the instrument is linear over our concentration range, the isovolume conversion can be made directly from the stopped flow equilibrium signals for $C_{iso} = 0.75$ and 1.5, and

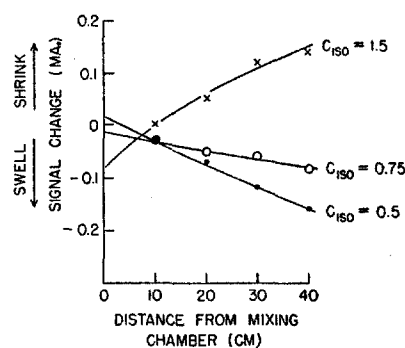


FIG. 8. Time course of volume changes in a typical experiment. The curves shown have been drawn by eye and have no theoretical basis.

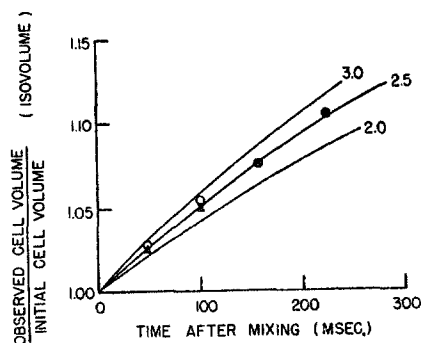


FIG. 9. Fit of the observed experimental data, after conversion to units of isovolume and time, to the theoretical curves. Theoretical curves (according to Equation 13) are shown for P_s values of 2.0, 2.5, and 3.0/sec. The experimental points represent three runs in the same experiment at $C_{iso} = 0.5$.

the measured isovolumes at these tonicities, which are 1.15 and 0.85 respectively (Fig. 7). Data from a typical experiment, converted to these units, are presented in Fig. 9, which represents the results of three runs in the same experiment on the same sample of blood at $C_{iso} = 0.5$.

P_s is next determined by superposing the time curves, calculated according to Equation 13, on the experimental data. The best fit is then chosen by eye, lining up the time scale exactly, but translating the isovolume zero to give the best fit. Curves have been calculated for P_s values of 1.0, 2.0, 2.5, 3.0, and

4.0/sec. In Fig. 9, the curves for P_s values of 2.0, 2.5, and 3.0/sec. are shown with the data, and it is clear that the value of 2.5/sec. represents the best fit to the data. Parenthetically, it might be observed that the fit of the curves to the data is almost completely independent of the value of W_{off} in the range of 0.46 to 0.72. The results of twenty determinations in eight experiments on young adults (seven male, one female (E. S.)) are presented in Table III.

The value of P_s is found to be 2.9 ± 0.3 /sec. The major component of the error results from the process of fitting the experimental data to the calculated curves. Since the interval between calculated curves is, in the region of interest, 0.5/sec., a value of ± 0.25 /sec. has been assigned to the scale factor error. The next most important source of error is the uncertainty in the diameter of the

TABLE III
Measurement of P_s

Experiment	Donor	Temperature °C.	P_s		
			$C_{iso} = 0.5$	$(\text{sec.})^{-1}$ 0.75	1.5
1	S. F.	24	3.0	3.0	3.0
2	T. R.	23	2.5	2.5	2.5
3	D. W.	24	2.5	3.0	3.0
4	E. S.	26	3.0	3.0	4.0
5	A. McF.	26	—	3.0	3.0
6	D. H.	25	—	3.0	3.0
7	T. R.	26	—	3.0	2.5
8	R. W.	26	—	3.0	3.0

Average (\pm standard deviation)..... 2.9 ± 0.3

tubing leading from mixing chamber to scattering chamber, which introduces a 9 per cent error. When these two errors are combined according to the usual rules, they yield an error of 0.36/sec., which is in reasonable agreement with the value of 0.34/sec., the calculated standard deviation of the results presented in Table III. This agreement suggests that the other sources of random error are relatively unimportant.

On the basis of the value for P_s , P_w may be calculated to have a value of 0.23 ± 0.03 (cm.⁴/osm., sec.). The unit is a measure of the cm.³ of water that will enter, in 1 second, 1 cm.² of cell surface exposed to a concentration difference equivalent to 1 osm./cm.³. The present figure is larger than that previously given by Jacobs (1), which when converted to the same units, is 0.13 to 0.15 (cm.⁴/osm., sec.). The difference is in accordance with Jacobs' (2) suggestion that his earlier values may have been too low.

A wide variety of other units have been used for the expression of P_w . Thus, the present value may be expressed in Jacobs' units as $0.095 \mu^3/(\mu^2, \text{atm., sec.})$,

or in Pappenheimer's (14) units as $0.92 \times 10^{-8} \text{ cm.}^3/(\text{cm.}^2, \text{ cm. H}_2\text{O, sec.})$. It can also be expressed in terms of a single red cell, as $1.5 \times 10^{-14} \text{ cm.}^3/(\text{red cell, cm. H}_2\text{O, sec.})$. These various alternative units differ only in respect to the units of length, and the choice of driving force; all show that the water enters very fast. If a 1 cm. H₂O pressure gradient were maintained across a single red cell, it would swell to double its volume in 0.54 sec.

In sum, a new method has been developed to measure the rate of entrance of water into a human red cell under an osmotic pressure gradient. The data thus obtained are essential to the determination of the "equivalent" pore radius of the human red cell (Paganelli and Solomon (15)). The further importance of the method lies in its ability to determine, for other cells as well as the human red cell, that physical constant which describes the major response of the cell to an applied osmotic pressure gradient.

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