

## PERMEABILITY OF THE HUMAN ERYTHROCYTE TO SODIUM AND POTASSIUM\*

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The permeability of the red blood cell to sodium and potassium has been the subject of many investigations in recent years but the results obtained are not definitive. Part of the confusion perhaps may be attributed to the fallacy of drawing generalizations from studies upon different species. Thus, while sodium is the principal base in the erythrocyte of the cat and the dog, potassium is the principal base in the erythrocyte of the rabbit, the monkey, and man. An impressive number of observations (1-10) suggests that erythrocytes are impermeable to base. An equally impressive number (11-20) suggests that red cells are permeable but only under conditions thought to be unphysiological. Yet a third group (21-28) supports the contention that permeability exists even within the physiological range. In the third group are the studies of Conway and Boyle (29) which suggest permeability to potassium but not to the larger sodium ion. Finally, there is the statement of Yannet, Darrow, and Cary (30) that erythrocytes are permeable to base only in species which have a preponderance of sodium in the cell. These workers affirm, however, that an experimental error as great as 30 per cent might distort their conclusions in regard to sodium permeability in those species which have a preponderance of potassium in the erythrocyte.

Recently, the use of radioactive isotopes of sodium and potassium

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has permitted a more exact evaluation of erythrocyte permeability. The collected data (31-35) on the rabbit, the dog, and man indicate that the erythrocyte of each is impermeable to potassium but permeable to sodium. The concentration of sodium in the erythrocytes of the rabbit and man, however, is so low that the deduction concerning permeability to this base may be in error.

The studies reported in this communication are in agreement with the studies on radioactive substances. The work was done on the blood from six healthy young persons. It was pursued as a control for similar studies on patients with pemphigus.<sup>1</sup> It is concluded that the human erythrocyte is permeable to sodium but not to potassium except under unphysiological conditions.

#### *Methods*

Approximately 80 ml. of blood were withdrawn from an arm vein and placed in a tube which contained sufficient heparin to prevent coagulation. No precaution against CO<sub>2</sub> loss was taken. Immediately after the blood was mixed with heparin, 2 ml. were pipetted into each of four flasks containing 20 ml. of isotonic sucrose (0.291 M), 40 ml. of isotonic sucrose, 20 ml. of isotonic saline (0.85 per cent), and 40 ml. of isotonic saline, respectively. The bloods were mixed with the suspension fluid, following which 2 ml. of the 1:11 suspensions and 4 ml. of the 1:21 suspensions were pipetted into 4 ml. hematocrit tubes. Also, 6 ml. of whole blood were pipetted into a 10 ml. hematocrit tube. All of the samples were centrifuged at 3000 R.P.M. for 3 minutes except the whole blood, which was centrifuged for 10 minutes. Essentially complete settling of the erythrocytes in the artificial media occurred during this time. The supernatant sucrose and saline were withdrawn by suction and as much plasma as possible was withdrawn from the tube containing whole blood. The latter was centrifuged for 40 minutes longer and the cell volume determined. In the first two experiments the remaining plasma was withdrawn, whereas in the other experiments the volume of the remaining plasma (less than 0.5 ml.) was determined and appropriate corrections for the sodium content of the plasma were applied to the analysis of the cell-plasma mixture so as to yield the cell sodium content.

<sup>1</sup> Kurnick, N. B., Lever, W. F., and Talbott, J. H., unpublished work.

All of the blood containers were placed in a constant temperature water bath at 40°. At intervals of 1, 2, 4, 6, 8, 14, and 24 hours, respectively, samples were withdrawn and the above procedure repeated.

The cells spun down from the sucrose and saline solutions were transferred quantitatively to quartz tubes and potassium determined by the modification of the method of Consolazio and Talbott (36) described elsewhere.<sup>1</sup> The cells obtained from the whole blood samples were transferred quantitatively to platinum crucibles, dried, and ashed with a few drops of concentrated sulfuric acid. The ash was dissolved in dilute hydrochloric acid and made up to a volume of 10 ml. Determinations of sodium and potassium were made upon aliquots of this solution according to the method described by Consolazio and Dill (37). The concentrations of sodium and potassium in the plasma were determined by the above methods. Water content was determined by desiccation at 110°.

### *Results*

The effect of volume changes is avoided in the analysis of the electrolytes of cells by the methods employed and any variation in concentration observed is, therefore, produced by exchange of electrolytes up to the time that hemolysis appears. This becomes apparent in from 2 to 4 hours in the dilute sucrose solution, in from 4 to 6 hours in the concentrated sucrose solution and in whole blood, and in 24 hours in the saline solutions. Once hemolysis appears, it progresses at a constant rate but in no sample except the sucrose suspensions did it exceed 10 per cent of the total cells.

The studies with the artificial solutions are similar to those described by Davson (14) and in general confirm his results. Thus, there is a rapid loss of potassium from the erythrocytes in sucrose solutions (Fig. 1). The rate of loss is somewhat greater than Davson observed but the temperature of equilibration was 15° higher than that used in his studies. Also, we observed a much slower rate of potassium loss in isotonic saline as compared with isotonic sucrose. A marked tendency toward erythrocyte agglutination in the sucrose suspension was confirmed. This phenomenon was interpreted by Davson as an effect of alterations in the erythrocyte membrane associated with permeability to

potassium. Our reason for using two dilutions of blood in sucrose and saline solutions was to test Davson's (14) conclusion that dilutions over the range 1:5 to 1:100 have no effect upon the rate of potassium loss. If this were correct, we would be entitled to regard any differences which might appear in base permeations

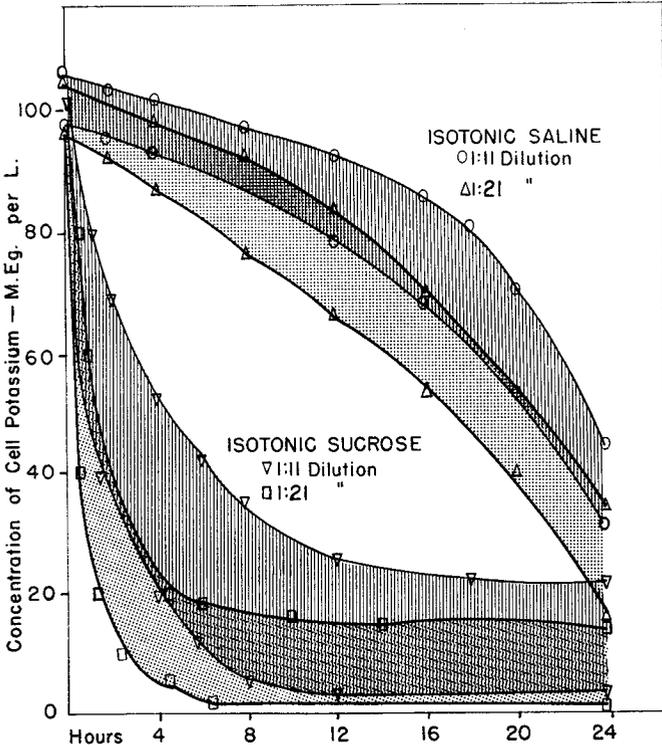


FIG. 1. Change in concentration of potassium per original liter of cells equilibrated at 40° with isotonic saline and isotonic sucrose. Each shaded area represents the range of six complete series of observations made at the various times indicated. The lines bordering each shaded area are drawn so as to give smooth curves.

in the blood of patients with pemphigus and in normal blood as caused by real differences in the state of the cell membranes and not due to original differences in the protective action of the serum due to its abnormal electrolyte concentrations. Fig. 1 indicates that even small amounts of serum exert a protective action, since

the rate of potassium loss is slightly greater in the more dilute solutions associated with earlier hemolysis and more marked agglutination. However, the difference between the two rates in each pair is small, so that direct comparison of the results in patients with pemphigus and normal subjects is justified.

The observations on potassium loss by the erythrocyte in whole blood incubated at 40° are presented in Fig. 2. The potassium content is corrected to 1 liter of original cells and the differences

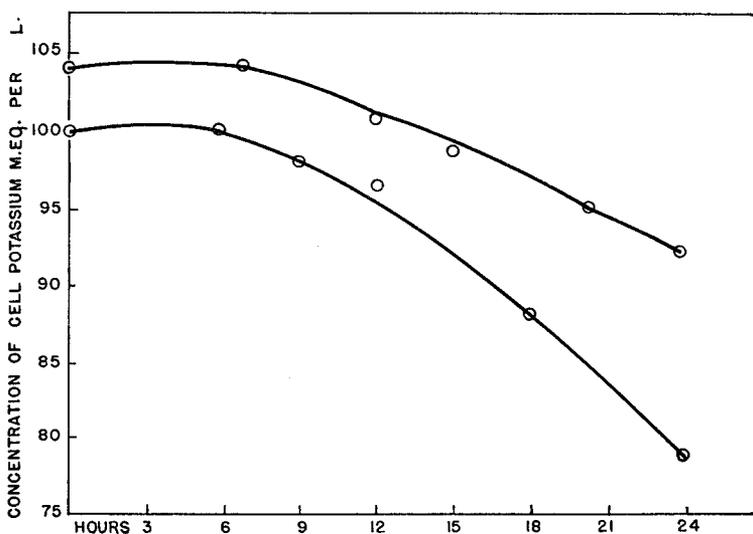


FIG. 2. Change in concentration of potassium per original liter of cells equilibrated at 40° in plasma. The area bounded by the curves represents the range of a series of observations on six bloods made at the various times indicated. The lines are drawn so as to give smooth curves.

in the original hematocrit do not appear. As in our other studies<sup>1</sup> the changes are real and independent of water exchange. It is apparent from the data that no loss of potassium occurs for the first 8 hours; thereafter, the loss is constant. Hemolysis also appears about this time but this alone does not account for the potassium loss. The hemoglobin content of the serum, as determined colorimetrically, demonstrates that hemolysis is responsible for approximately 50 per cent of the potassium exchange.

The cell sodium studies are summarized in Fig. 3. The observa-

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tions are corrected to an original volume of 1 liter of cells and the changes are absolute. The data indicate that sodium enters the cells from the plasma at a slow, constant rate. No correction for

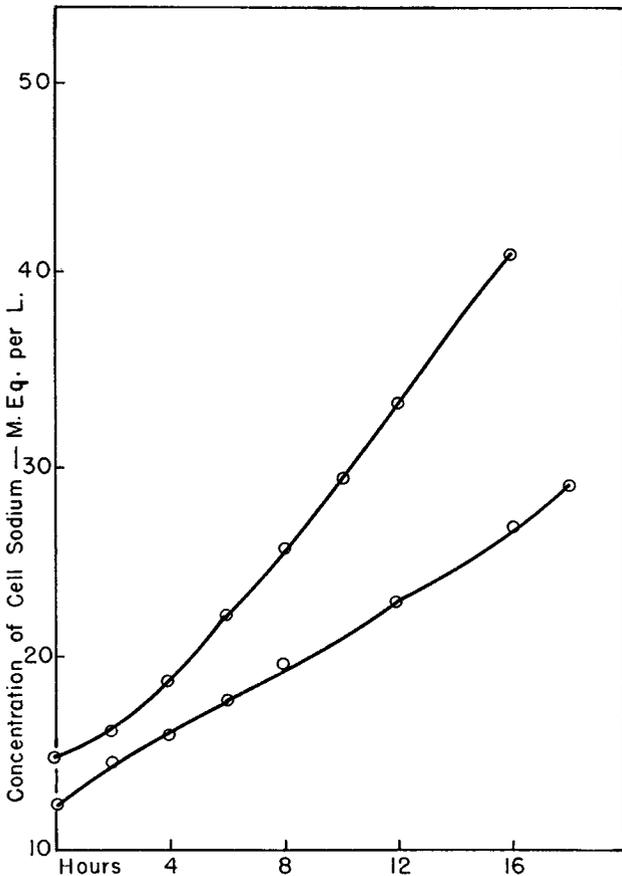


FIG. 3. Change in concentration of sodium per original liter of cells equilibrated at  $40^{\circ}$  in plasma. The area bounded by the curves represents the range of a series of observations on six bloods made at the various times indicated. The lines are drawn so as to give smooth curves.

hemolysis is made. If this were done, the rate of sodium gain would be increased over that shown.

The concentrations of plasma constituents were determined because it was hoped that they would serve as a check upon the

cell constituents. Before these data are given, however, it seems desirable to consider water exchange between cells and plasma, inasmuch as the plasma analyses were made upon 1 ml. amounts of plasma after equilibration. Water changes were determined from hematocrit readings, plasma water contents, and serum solid contents and calculated from the observed concentrations of sodium and potassium in the plasma, and the observed changes in the cellular content of these bases. The hematocrit readings are given in Table I. The trend in the cell volume is believed to be more significant than the absolute values. It is apparent that the cell swells in spite of hemolysis.

TABLE I

*Cell Volume of Whole Blood after Incubation for Various Periods of Time and Centrifugation at 3000 R.P.M. for More Than 40 Minutes*

The values are expressed in per cent.

Time	Subject					Hemolysis
	N. B. K.	R. S. M.	A. B.	W. L.	E. J.	
20 min.....	45	48	42	46	42	None
1 hr., 25 min.....				47	42	"
2 hrs., 20 ".....			40	48	42	"
4 " 20 ".....		53	41	48	43	Slight
6 " 20 ".....			41	49	44	"
8 " 20 ".....	62*	52	43	50	44	"
15 ".....				53	47	Moderate
25 ".....	55			55		Marked

\* Doubtful value.

Direct determinations of plasma water (Table II), which was studied in two experiments only, proved unsatisfactory because of the small volume of plasma available for analysis. Open containers were used and the desiccated serum was used later for determination of sodium and potassium. The loss of water is apparent. The increase in protein content in the same sample of plasma is confirmatory of the water shift. The determination of total solids per liter of plasma is not affected by the use of open containers and, therefore, is more reliable. In Fig. 4, data from three experiments are given. The gain in content of solids amounted to more than 10 per cent in 14 hours. Hemolysis contributed in a small measure to this change.

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The plasma sodium and potassium observations are presented in Figs. 5 and 6. An initial increase in plasma sodium concentra-

TABLE II  
Water and Total Nitrogen Content of Plasma Incubated with Red Blood Cells

Time	Water		Total nitrogen	
	Subject E. J.	Subject W. L.	Subject E. J.	Subject W. L.
	<i>gm. per l.</i>	<i>gm. per l.</i>	<i>gm. per 100 cc.</i>	<i>gm. per 100 cc.</i>
20 min.....	924	927	13.20	10.76
1 hr., 25 min.....	928	919		
2 hrs., 20 ".....	918	924		
4 " 20 ".....	916	918	13.43	
6 " 20 ".....	910	917		
8 " 25 ".....	918	926	14.41	
16 ".....	914	924		11.95
25 ".....		921		

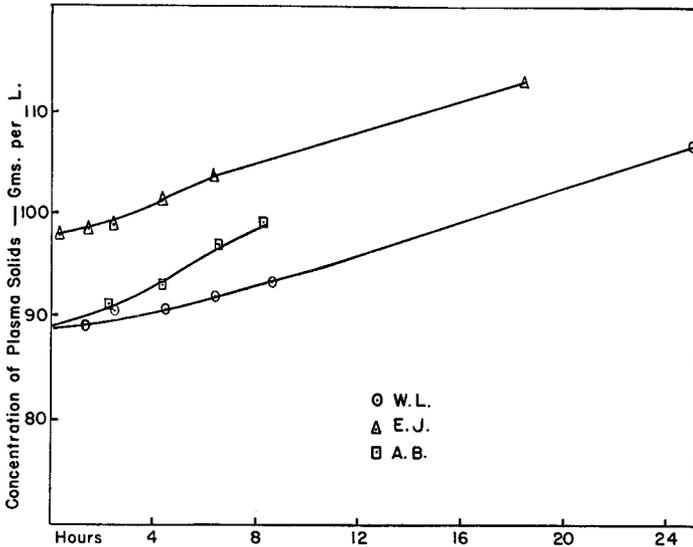


FIG. 4. Change in concentrations of solids in plasma equilibrated at 40° with cells.

tion appeared during the first 4 to 8 hours. Thereafter the plasma lost sodium rapidly. The apparent contradiction to the cell so-

dium data is explained when it is appreciated that the effect of water changes is excluded only in the cell analysis. A steady trend toward concentration of plasma solids has been noted, indicating a constant transfer of water from serum to cells. If this

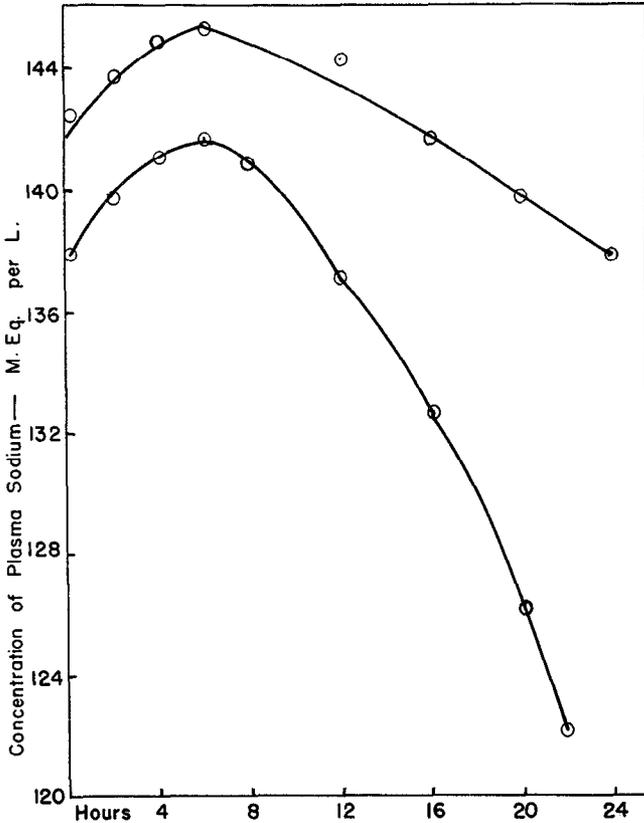


FIG. 5. Change in concentration of sodium in plasma equilibrated at  $40^{\circ}$  with cells. The area bounded by the curves represents the range of a series of observations on six bloods made at the various times indicated. The lines are drawn so as to give smooth curves.

correction were applied to the plasma curves, its effect would be to change the initial increase to a very slight decrease.

There is no increase in plasma potassium until 4 hours have elapsed. The effect on the shape of the curve during the first 4

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hours, if corrected for water exchange, would be negligible. Later, correction would tend to reduce the rate of increase and would make it coincide with the rate of cell potassium change. Correction for hemolysis would be in the same direction as for water shift.

From the data on absolute changes in sodium and potassium in the cells and concentration changes in the plasma, it is possible

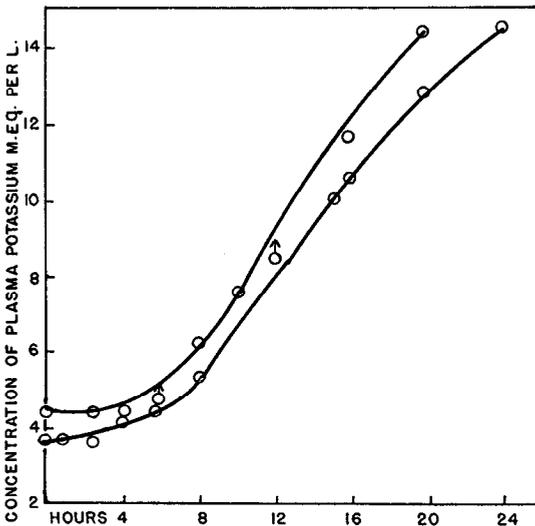


FIG. 6. Change in concentration of potassium in plasma equilibrated at 40° with cells. The area bounded by the curves represents the range of a series of observations on six bloods made at the various times indicated. The lines are drawn so as to give smooth curves.

to calculate the rates of water exchange quantitatively by the formula,

$$\frac{V_t}{V_0} = \frac{(B)_0 + B}{(B)_t}$$

where  $V_t$  represents the final volume of a given original volume of plasma ( $V_0$ ) at time  $t$ ,  $(B)_0$  and  $(B)_t$  represent the base concentrations in the original and final plasma, respectively, and  $B$  (which is assumed to occupy no space) represents the absolute increase in the base  $B$  per volume of original plasma. When  $V_0 = 1$  liter, the final volume per liter of original plasma is obtained. The

results of the calculations except those of the first two experiments are presented in Fig. 7.

Calculation of  $V_t$  for potassium introduces too large an error to be of value. An example will illustrate this. If  $K_{c0} = 100 \pm 2$ ,

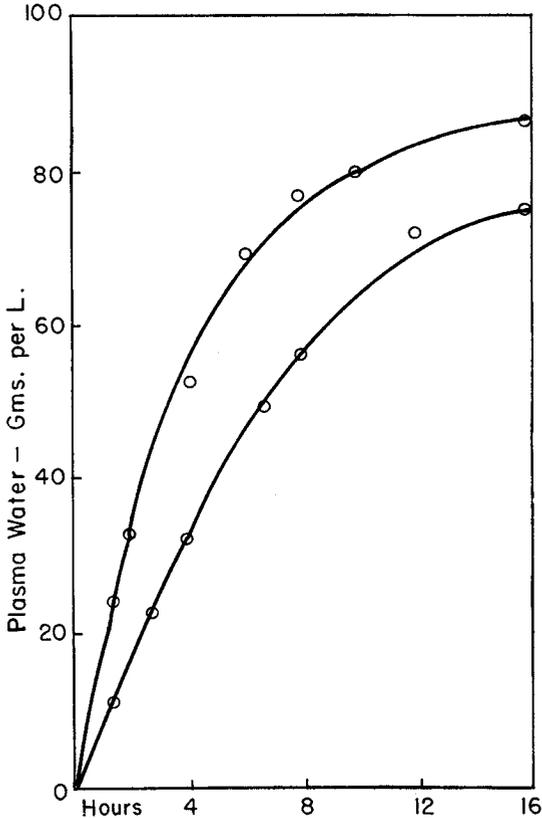


FIG. 7. Change in concentration of water per original liter of plasma equilibrated at  $40^\circ$  with cells. The area bounded by the curves represents the range of a series of observations on six bloods made at the various times indicated. The lines are drawn so as to give smooth curves.

$K_{c24} = 90 \pm 2$ ,  $K_{s0} = 4$ ,  $K_{s24} = 15$ , and  $V_c = 50$  per cent, then  $K = 10 \pm 4$  at 24 hours, and  $V_{K24} = (4 + 10 \pm 4)/16 \times 1000 = 875 \pm 250$  ml. Thus the calculated volume change might vary from  $+125$  to  $-375$ . Such large errors would not appear if

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sodium data were substituted. If  $\text{Na}_{c0} = 13 \pm 1$ ,  $\text{Na}_{c24} = 40 \pm 1$ ,  $\text{Na}_{s0} = 140$ ,  $\text{Na}_{s24} = 130$ , and  $V_c = 50$  per cent, then  $V_{\text{Na}24} = 1000 \times (140 - 27 \pm 2)/130 = 870 \pm 15.4$  ml. Our calculations therefore have been confined to sodium. (The subscripts refer to phase and time. Thus,  $K_{c0}$  represents the concentration of cell K in milliequivalents per liter at zero time of drawing blood;  $K_{c24}$  represents the content of K in milliequivalents of the same 1 liter of cells after 24 hours of equilibration;  $K_{s0}$  and  $K_{s24}$  represent the plasma concentrations at the respective time intervals.)

The effect of hemolysis is to increase the plasma volume values at the expense of the cell volume. However, the same trend toward reduction of  $V_s$  is observed as is found in the hematocrit determinations and is more constant because of the greater accuracy of the method. Had there been no hemolysis, there would have been no reduction in the rate of this volume shift, such as occurs after 12 hours.

### DISCUSSION

The analysis of potassium loss by the erythrocyte confirms the results of Davson (14), Kerr (17), Maizels (18), and others in demonstrating the injurious effect produced upon the erythrocyte membrane by suspension in non-electrolytic solutions and to a lesser degree in isotonic saline solutions. Even small amounts of plasma appear to exert a slight protective effect in reducing the rate of potassium loss. In undiluted plasma there is no loss of potassium for the first 8 hours, at which time hemolysis appears. While the degree of hemolysis is insufficient to account for all of the potassium lost, it is believed that permeability to potassium exists only under abnormal conditions which permit hemolysis and agglutination. It might be argued that the permeability to potassium observed after 8 hours in the whole blood studies is not indicative of a degenerative change in the erythrocyte membrane but is due to the water shift which became large enough to stretch the membrane "pores" (38) to a critical size except for the potassium ion. There are two reasons for our inability to subscribe to a simple "pore" theory. (1) We have observed a similar loss of potassium in patients with pemphigus who exhibit a different magnitude of water transfer;<sup>1</sup> (2) permeability to the larger sodium

ion exists from zero time. It is concluded that the water shift merely contributed to the alteration of the cell membrane and that potassium permeability exists only in the altered state.

The observations on sodium, on the other hand, reveal a different process. Permeability to sodium begins promptly and several hours before hemolysis appears. This is observed in patients with pemphigus<sup>1</sup> as well as in normal controls and makes it unnecessary to postulate any basic alteration in the erythrocyte membrane in this malady. The size of the water shift is sufficient to account for the conclusions of the first group of authors (1-10) whose determinations are based on volume changes during carbon dioxide equilibration experiments without direct chemical analyses of the cell electrolytes. They concluded that permeability to base does not exist. Eisenman, Hald, and Peters (39) from *in vivo* experiments stated that, "On the whole the red cells in the circulating blood seem to react as they do in the test-tube, expanding and contracting in response to osmotic influences by exchanges of water without base." However, their chemical analyses demonstrated transfer of base. They suggested, because of other data from their laboratory (40) which indicated phosphorus permeability only when the cells were active metabolically, that the cell membrane is, in fact, *impermeable* to base, but that base nevertheless does traverse the cell membrane in response to metabolic requirements. It is our opinion that metabolic forces may affect the point of ionic equilibrium, but cannot alter permeability (unless it be assumed that a complete alteration in the nature of the membrane is produced by them). We regard permeability as a function of the membrane alone, while the point of equilibrium is determined by forces acting on both sides of the membrane (including metabolic as well as osmotic forces) which impel permeable ions to traverse the membrane. In such a theory, we explain the migration of phosphorus only across the membrane of metabolically active cells by assuming that the membrane is permeable to phosphates under all conditions, but that permeation is detectable only when the equilibrium is disturbed by the metabolism of phosphates. We are able, also, by this theory, to account for the osmotically abnormal distribution of sodium between cells and plasma in exacerbations of pemphigus.<sup>1</sup> Furthermore, wide variations in cell base which were observed by Hald and Eisenman (41) and offered as proof of their

contention have not been observed in other laboratories (42, 43).<sup>1</sup> The data of Butler and MacKay (44) are in accord with our conclusions. They observed a tendency for the cell sodium to decrease when the Na:K ratio in the diet was reduced; meanwhile the cell potassium content remained constant.

## SUMMARY

It is concluded that the reestablishment of osmotic equilibrium upon equilibration of whole blood at a  $p\text{CO}_2$  far below that encountered in the body is attained not only by an exchange of anions and water between cells and plasma but also by an exchange of sodium. The intact erythrocyte membrane is impermeable to potassium but not to sodium. Migration of base, therefore, is not merely a mechanical transfer through "pores" in the cell membrane. The erythrocyte membrane becomes permeable to potassium following injury.

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