Red Cell Membrane Structure and Ion Transport

A. K. SOLOMON

From the Biophysical Laboratory, Harvard Medical School, Boston

The red cell in man is a biconcave disk, some 8.4 μ in diameter, 2.4 μ thick at its widest portion, and 1 μ thick at its narrowest. It is bounded by a membrane primarily composed of protein and lipid, the latter sufficient in amount to cover the red cell with a layer two molecules thick. The lipid layer is bounded on the outside, and possibly on the inside, by a layer of protein. The thickness of the membrane is not known accurately since the most reliable studies, those made with the electron microscope and by optical refraction, are necessarily made with dried material. The best estimates of membrane thickness lead to values of the order of 100 Å. Within the human red cell, there is a high K concentration, 143 mM/liter cell water, and a low Na concentration, 13.9 mM/liter cell water. In the environment, the situation is reversed, since plasma contains 5.0 mM K and 154 mM Na/liter plasma water (1–3). The present paper is concerned with the role of the cellular membrane in the creation and maintenance of these sharp differences in ionic composition across a barrier that is only a few molecules thick, a problem common to a wide variety of living cells. The conclusions are based on studies carried out in this laboratory in company with a number of associates, including R. Villegas, D. H. P. Streeten, V. Sidel, C. V. Paganelli, D. Goldstein, G. L. Gold, T. J. Gill, J. Evans, and T. C. Barton.

The passage of alkali cations across the red cell membrane is very slow, 3.1 mM Na and 2.1 mM K/liter cell hour (1, 2). In the case of the principal intracellular cation, K, this corresponds to a half-time for exchange of 31 hours. In contrast, Cl, the principal anion, enters the red cell freely and exchanges with a half-time of 0.24 seconds (4). Since the electrophoretic mobilities of these two ions are closely similar, this difference is commonly interpreted in terms of a positive charge barrier in the channels which connect the interior of the cell with its environment. The charge density required to produce this 5×10^5–fold difference in rates of entrance may be calculated approximately according to the equations given by Meyer and Sievers (5). This calculation is independent of the dimensions of the channels through which the ions are presumed to pass, and leads to a charge density within the channels of 106 M/liter. Since water is present at a concentration somewhat less than 55 M/liter, the charge density of 106 M/liter is clearly impossible of achievement. However, as Sollner has pointed out (6), in the limit when the channels are small enough, a single positive charge will suffice to block the

pores completely to positive ions. Thus the charge density calculation leads to the conclusion that the channels through the cellular membrane must be small, probably of the order of magnitude of the hydrated ionic radii of the K and Cl ions.

Though a system of small charged channels will suffice to account in a qualitative way for the difference between the rates of K and Cl entrance into the cell, it will not account for other known facts concerning K entrance. The K concentration within the cell is some 28 times greater than that without, and the potential difference across the membrane is far too small (1) to account for this large deviation from equilibrium. The behavior of K may be contrasted with that of Cl, for which the concentration and potential gradients are characteristic of equilibrium. Entrance of K into the cell in-

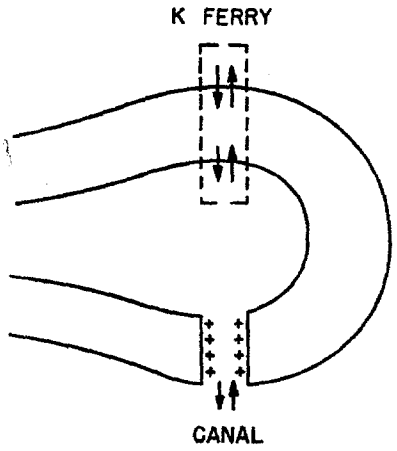

FIGURE 1. Schematic representation of K flux in human red cells.

volves transport up an electrochemical potential gradient, an active process which requires energy. It is commonly assumed that several steps are involved, as indicated diagrammatically in Fig. 1. K entry makes use of the K ferry, a lipid-soluble molecule which is confined to the membrane phase. The movement of this carrier across the membrane, either when complexed with K or on its return journey, must be coupled to a supply of energy derived from ATP. The major route of exit from the cell is through the canal, which is assumed to be lined with positive charges as already discussed. Movement through the canal is predominantly down the concentration gradient, a process which requires little or no energy.

It seems reasonable to assume that all the steps in the chain are reversible, as is the usual case for chemical reactions and for diffusion. Thus, the association of K with the carrier on the outside of the membrane and its dissociation on the inside, are both considered to be reversible reactions, though the equilibrium favors inward movement. Furthermore, some K ions can enter

the cell by diffusing upstream in the canal, but these ions are considered to comprise only 5.5 per cent of those that leave by downstream diffusion.

Although a hypothesis of this general type is widely accepted, evidence in its favor is almost entirely inferential. We have been concerned with attempts to put it on a firmer base by experiments designed to verify the following three statements: (*a*) pores of a size comparable with the hydrated ionic radii of K and Cl exist in the red cell membrane; (*b*) the major route of K entrance into the cells is by a route different from that taken by water; and (*c*) the major route of K exit from the cells is along a path different from that used for entrance. It would also be particularly important to demonstrate the reality of the ferry by isolation of the molecule or molecules involved but this has not yet proved possible, either in the red cell or other cells. In the human red cell, the density of ferry terminals on the outer surface has been calculated (7) to be 1 per million $Å^2$ of cell surface, a very small population indeed. Furthermore, if each terminal is serviced by a single ferry, and each ferry takes only a single K ion, the half-time for the existence of the K ion—ferry complex is 0.2 second. In consequence, it would seem most difficult to extract pure carrier substance from the red cell membrane. Even if the number of ferries per site were some orders of magnitude larger, the problem of extraction and identification of the carrier molecules would still be formidable.

Existence of Pores

Measurement of the rate of passage of water through the cellular membrane provides information which can be used to demonstrate that water-filled channels exist in the red cell membrane. When water crosses a cellular membrane by the process of diffusion, the rate per unit concentration difference is proportional to $A_w/\Delta x$, in which A_w represents the area through which water can diffuse, and Δx represents the path length through the membrane. On the other hand, when water enters the cell under an osmotic pressure gradient, the rate per unit pressure difference is proportional to $A_w\ r^2/\Delta x$, in which r is the pore radius of the channel, assumed circular, through which the water enters. The derivation of these equations, and the assumptions operative in such a calculation have already been discussed in detail (8–11). In the human red cell, the permeability to water under a diffusion gradient (11) is 0.62×10^{-14} ml. H_2O/(sec., cm. H_2O pressure, red cell), while that under an osmotic gradient (12) is 1.5×10^{-14} ml. H_2O/(sec., cm. H_2O pressure, red cell). Since these values, expressed in the same units, are different though the driving forces are equal, some process other than diffusion must be involved in the osmotic gradient experiments. This

process, as pointed out by Koefoed-Johnsen and Ussing (8), is bulk flow; hence water-filled channels connect the interior of the cell to its exterior environment.

These two permeability coefficients may be used to calculate the equivalent pore radius which for the human red cell is 3.5 Å. Characterization of a membrane in terms of an equivalent pore radius does not imply that the pores are rigid or fixed in space; rather the *equivalent* pore radius is the radius

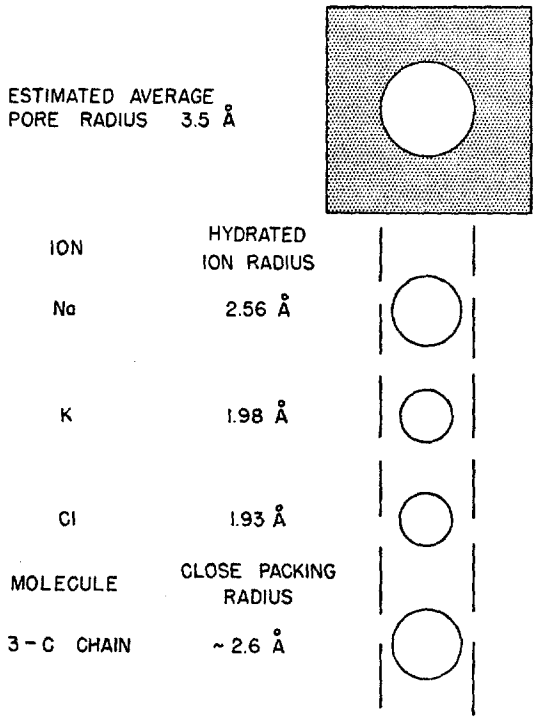

FIGURE 2. Schematic representation of red cell pore. The hydrated ionic radii have been obtained from conductance data by Gorin (14).

that would obtain for an ideal membrane whose permeability to water is the same as that of the human red cell. Similar determinations in other species (13) lead to equivalent pore radii of 4.1 and 7.4 Å for the red cells of beef and dog, respectively. Fig. 2 shows a comparison of the equivalent pore radius in man with the hydrated ionic radii of a number of molecules. The fit for K and Cl is relatively tight, so that the charge density within the pores may be expected to fall within reasonable limits. It has long been known that the permeability of human red cells to a variety of non-lipid–soluble non-electrolytes falls off sharply when the size of the probing molecules ex-

ceeds 3 carbons. This is also in qualitative agreement with the 3.5 Å equivalent pore radius.

An independent approach to the problem is afforded by a quantitative investigation of the restriction offered by pores to the passage of small lipid–insoluble, non-electrolyte molecules. As the size of the probing molecules becomes larger and larger, the rate of diffusion through the pore decreases; a quantitative theory exists which relates the diffusion permeability to the equivalent radius of the pore. The customary method of measuring diffusion into red cells is by observation of the rate of cell swelling due to the water which follows the solute into the cell. The photometric measurements employed make possible accurate measurement of rates for processes which may be essentially complete in a matter of seconds. Conventional mathematical treatment of the data thus obtained requires knowledge of the osmotic pressure developed when the cells are suddenly immersed in a solution of the probing molecules. But this is precisely the most difficult knowledge to obtain since, as Staverman (15) has pointed out, the osmotic pressure developed across a leaky membrane is not given by the theoretical van't Hoff equation. In consequence, it is necessary first to measure the osmotic pressure that is actually developed for each of the solutes whose diffusion is to be measured.

Diffusion is described by Fick's law which states that

$$dn/dt = -(D_s A_{sd}/\Delta x)\Delta C$$

in which: n is the number of probing molecules in the external solution, t is time, D_s is the diffusion coefficient for the probing molecule in free solution, A_{sd} is the apparent pore area available for diffusion, Δx is the path length through the membrane, and ΔC is the concentration difference across the membrane. This formulation ascribes all the resistance to free diffusion to $A_{sd}/\Delta x$ so that the determination of this parameter is the object of these experiments. Similar experiments may be carried out in which solutions are filtered through membranes. Under these conditions, the parameter which is measured is related to $A_{sf}/\Delta x$, in which A_{sf} is the apparent pore area available for filtration.

It can be shown that the measured osmotic pressure across a leaky membrane is directly related to the apparent pore area for filtration. Staverman has described the osmotic pressure developed across such a membrane in terms of the "reflection coefficient," σ, which is defined by $\sigma = \pi_{obs}/\pi_{theor}$; π_{obs} is the osmotic pressure actually observed and π_{theor} is the theoretical van't Hoff osmotic pressure (after correction by the osmotic coefficient). In an ideal semipermeable membrane, $\sigma = 1$, whereas in a membrane with pores so large that it cannot discriminate between solute and solvent, $\sigma = 0$, and no

osmotic pressure is developed. Durbin, Frank, and Solomon (16) give the following equation to relate σ to filtration pore areas,

$$\sigma = 1 - A_{sf}/A_{wf}$$

in which A_{wf} is the apparent pore area for the filtration of water through the membrane. This relationship has been confirmed experimentally by Durbin (17). $A_{sf}/\Delta x$ is given by

$$\frac{A_{sf}}{\Delta x} = (1 - \sigma)\frac{A_{wf}}{\Delta x}$$

$A_{wf}/\Delta x$ may then be obtained from the value of $A_w/\Delta x$ previously determined for human red cells by Paganelli and Solomon (11), and their Equation 20.

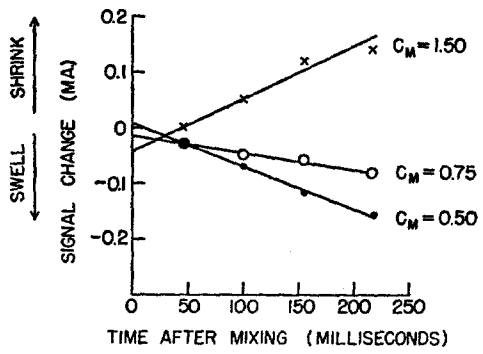

FIGURE 3. Time course of volume changes in human red cells exposed to anisosmolar solutions. C_m refers to the relative osmolarity of the solution to which the cells are exposed (C_m = 1 for an isosmolar solution). The points are experimental and the lines have been drawn by eye.

When a red cell is immersed in a solution, water will immediately begin to move into or out of the cell, depending on the osmotic pressure gradient. If the solution contains a penetrating solute, this initial water movement will be modified as the solute begins to diffuse into the cell. If, however, at zero time, there is no tendency for the water to move in either direction, the cell is in osmotic equilibrium, the osmolar concentration of its contents exactly and instantaneously balancing that of the environment. Thus, osmotic equilibrium is characterized by zero rate of cell swelling at zero time. The apparatus developed by Sidel and Solomon (12) permits measurements of cell volumes as rapidly as 50 milliseconds after the cell has been exposed to its new environment so that zero time determinations involve only a short extra-

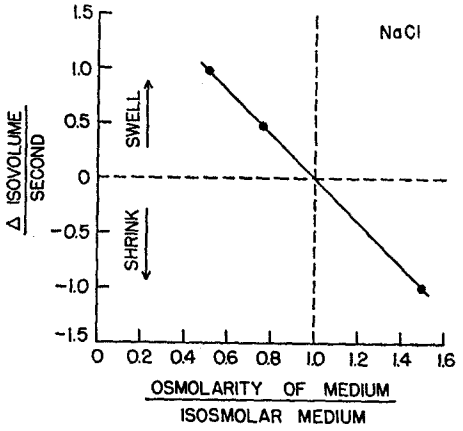

FIGURE 4. Initial slope of cell swelling as a function of osmolarity of medium when cells are exposed to solutions in which NaCl concentration alone is varied. Δ isovolume per second gives the rate of change of cell volume at zero time, expressed as a fraction of the initial volume.

polation. Fig. 3 shows the volume changes observed by these authors when human red cells are exposed to anisosmolar salt solutions. The changes in osmolarity were achieved by the addition or subtraction of NaCl in a normal red cell buffer solution. Since human red cells are effectively impermeable to NaCl, the initial rate of cell swelling should be zero when the solution is isosmolar, as measured by freezing point depression. Fig. 4 shows that this

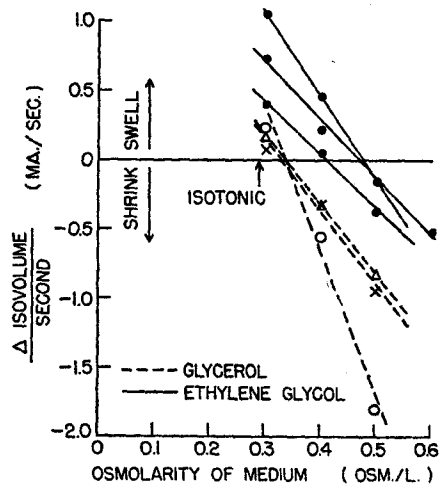

FIGURE 5. Initial slope of cell swelling when red cells are exposed to non-lipid-soluble non-electrolyte solutions. Three separate experiments were carried out with glycerol and with ethylene glycol.

requirement is satisfied. However, when the cell is exposed to penetrating solutes, such as glycerol[1] and ethylene glycol, higher concentrations of solute are required to achieve effective isosmolarity, as shown in Fig. 5.

$A_{sf}/\Delta x$ obtained in this way may now be compared to the predictions of

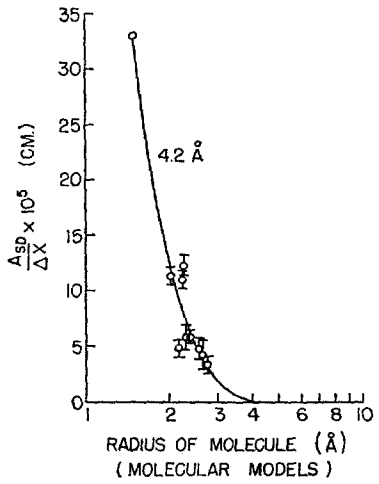

FIGURE 6. Equivalent pore radius in human red cells; $A_{sd}/\Delta x$ as a function of molecular radius. The points are derived from experimental data ($A_{sd} = A_{sf}/[2 - (1 - a/r)^2]$) and the height of the vertical lines shows the standard error of the mean. The point for water, which serves to normalize the curve, has been taken from Paganelli and Solomon (11) and is shown with no error. The theoretical curve has been drawn for an equivalent pore radius of 4.2 Å according to Equation 11 of reference 19. It can be shown that this procedure is identical with that described in the text using Equation 19 of reference 19. The radius of the molecule has been determined from molecular models as described in the text. The molecules used are: acetamide, propionamide, malonamide, urea, thiourea, methylurea, ethylene glycol, propylene glycol, and glycerol.

the theory of restricted filtration. Dividing the equation for restricted filtration given by Renkin (19) by Δx, we obtain

$$\frac{A_{sf}}{\Delta x} = \frac{A_p}{\Delta x}\left[2\left(1 - \frac{a}{r}\right)^2 - \left(1 - \frac{a}{r}\right)^4\right]\left[1 - 2.104\left(\frac{a}{r}\right) + 2.09\left(\frac{a}{r}\right)^3 - 0.95\left(\frac{a}{r}\right)^5\right]$$

in which A_p is the geometrical pore area, r the equivalent pore radius, and a the radius of the probing molecule. These molecular radii have been obtained by constructing accurate models of the molecules and measuring the

[1] It should be pointed out that this method of measuring the effective pore area for filtration is based upon measurements made at zero time, before the solute molecule has penetrated the membrane. Thus the fact that $A_{sf}/\Delta x$ for glycerol is normal as compared with other small lipid-insoluble molecules does not constitute evidence contrary to the suggestion of Stein (18) that the N-terminal groups of histidine are involved in glycerol diffusion into human red cells.

dimensions of the model. The radius is taken as $a = 0.5 \sqrt[3]{d_1 d_2 d_3}$ in which d_i is the diameter of the model taken along three mutually perpendicular axes. r may be obtained graphically as has been done in Fig. 6. It will be seen that a single curve, drawn for an equivalent pore radius of 4.2 Å fits all the experimental data obtained. This would suggest that the pores are characterized by a *single* mean equivalent radius, and therefore form a homogeneous population. This behavior for a single cell membrane may be contrasted to the results obtained with a complex tissue, the gastric mucosa, in which two distinct and separate mean equivalent radii were required in

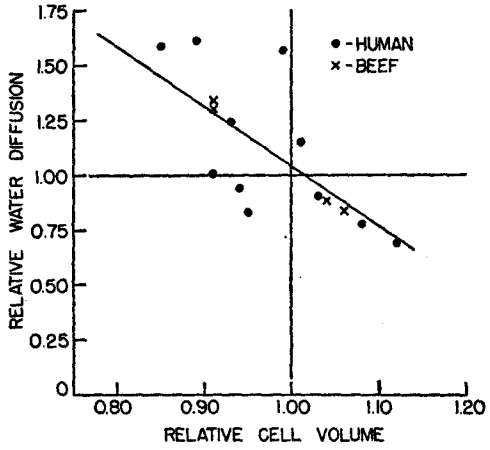

FIGURE 7. Dependence of water diffusion on relative cell volume.

order to describe the data. It is indeed gratifying that the 4.2 Å equivalent pore radius obtained by the restricted filtration method is in such good agreement with the value of 3.5 Å previously deduced from the measurements of water fluxes. Such close correlation supports the validity of the extension of Poiseuille's law to water flow through pores of small dimensions.

Does K Enter the Cells by the Same Route as Water?

It has been shown by Villegas, Barton, and Solomon (13) that the rate of water diffusion into beef red cells is a function of the osmolarity of the medium in which the cells are suspended. We have recently found that human red cells behave similarly, as shown in Fig. 7. The effect is quite marked, since a 10 per cent increase in cell volume results in a 28 per cent *decrease* in the rate of water diffusion. Villegas *et al.* ascribed the effect to swelling produced within the cellular membrane itself, which is considered to swell as the cell swells. Since the area of the red cell membrane has been shown by Ponder (20) to remain constant over such small changes in cellular volume, swelling

of the membrane is presumed to result in compression of the pores. Since the pores are small, a slight change in effective pore radius should cause a very large change in water diffusion, in agreement with the results shown in Fig. 7.

This observation makes it possible to compare the route of K entrance into cells with that taken by the water. If they both enter by the same route, a 10 per cent change in cell volume should affect K influx at least as much as water entrance by diffusion. In preliminary studies, we have found the

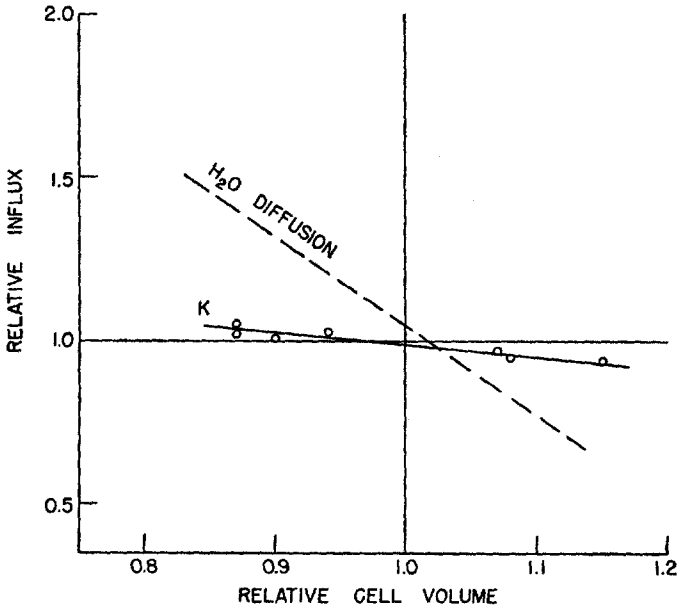

FIGURE 8. Dependence of K influx on relative cell volume. The dashed line for H_2O diffusion is taken from Fig. 7.

effect of cell swelling on K influx to be very small, as shown in Fig. 8. These results lead to the conclusion that the K ions do not enter the cell by the same pathway taken by the water. This conclusion is independent of the nature of the hypothesis used to explain the influence of osmolarity on water diffusion.

Further evidence that separate pathways are involved may be obtained from a comparison of the number of channels used by the water with the number of sites effective for the inward transport of K. $A_w/\Delta x$ is given by Paganelli and Solomon (11) as 3.3×10^{-4} cm. If the membrane is about 100 Å thick, in accord with recent electron microscope measurements, and if the path length through the membrane may be equated with the membrane thickness, A_w will be 3.3×10^{-10} cm.^{2}, or 0.02 per cent of the total cell surface.

On the basis of a 3.5 Å radius pore, there will be about 0.9×10^5 pores/cell. This number may be compared with the number of sites effective for K influx, as determined from the studies of the inhibition of K influx by ouabain. Solomon, Gill, and Gold (7) have estimated that there are less than 1.2×10^4 sites per cell for K influx, and Glynn (21) has independently concluded that the number lies between 1 and 3×10^3. Thus the number of sites is smaller than the number of pores by at least one order of magnitude, so that it would seem unlikely that the pores could serve as sites, unless only a small fraction of them were effective at any single time.

We might also ask whether the complete cellular membrane is required for the K influx process. Lovelock (22) has shown that treatment of human red cells with alumina causes the membrane to lose a large part of its lipid. We have exposed red cells to alumina under conditions which should cause the loss of 28 per cent of the lipoprotein, 15 per cent of the cholesterol, and 8 per cent of the phospholipid. Although this treatment results in some hemolysis, treated cells are still able to transport K. Influx remains closely equal to efflux, but the flux rates increase by about 20 per cent, not only for K, but also for Na. At the same time, glucose consumption increases 2.9 times. This would suggest that the K sites do not lie on the outermost surface of the cellular membrane, or conversely that an appreciable fraction of the membrane is superfluous to the maintenance of cellular integrity and intracellular function. The great increase in glucose consumption may possibly be ascribed to a greater ease in reaching metabolic sites—as if the underbrush were cleared away. The most effective inhibitor of K influx, ouabain, is presumed to act directly on the K influx sites (7). The effect of this inhibitor is independent of the alumina treatment, as would be expected if the lipids removed were not essential to K influx.

Is the Efflux Canal Separate from the Influx Ferry?

The heavy metal, lead, has been shown to cause a considerable loss of K from red cells (23–27). We have studied the effect of small doses of lead on K flux in both directions. In 6 experiments at 37°C. in which the Pb concentration in the plasma was 10^{-4} M, the K efflux rose from a control value of 1.63 mM K/liter cell hour to 8.17 mM K/liter cell hour. This fivefold increase in K efflux shows that the heavy metal acts on the efflux process. If the action is solely to increase the permeability of the canal, some slight effect should also be expected on K influx, since as previously pointed out, a small number of K ions can enter upstream through the canal. The experiments indicate that the increase in influx that may be attributed to Pb action is 25.6 per cent of the increase in efflux. On theoretical grounds the increase in influx should have been only 5.5 per cent of the increase in efflux. Thus Pb in this con-

centration may be considered to act primarily, but not solely, on K efflux. On the basis of these experiments, we may conclude that the efflux goes by a different route than does the influx. The evidence from the Pb experiments, however, is not sufficient to identify the canal as the major route of K efflux; the findings are compatible with this conclusion, but do not prove it.

For some time we have speculated on the origin of the positive charge barrier within the canal. It has seemed particularly attractive to assume that the barrier was provided by Ca ions. Winkler and Bungenberg de Jong (28, 29) have suggested that Ca is an essential component, required for the stability of the red cell membrane. Walters (30) in our laboratory has devoted much effort to quantitating the Ca content of red cell membranes. The high

TABLE I

REPLACEMENT OF INTERNAL K BY VARIOUS EXTERNAL CATIONS* AFTER 24 HOURS' EXPOSURE AT 4°C. (32)

External cation	Loss of K	Gain of external cation
	per cent	*per cent*
Ca	95	85
Ba	62	55
Mg	55	40
Sr	50	46
Na	32	33

* The solutions of the chlorides of the alkaline earths show some pH differences. In 0.1 M concentration, the pH's are: $CaCl_2$, 7.8; $BaCl_2$, 5.0; $MgCl_2$, 5.0; and $SrCl_2$, 5.5.

content of iron within the cell has made it difficult to obtain good analytical data, but his results suggest that the Ca content of human red cell membranes lies between 0.4 and 1 mM/liter cell.

In some of our earlier experiments (31), blood was drawn through a resin column to remove Ca, thus preventing clot formation. It was occasionally observed that the intracellular K would fall by as much as 1 to 2 mM/liter cell during passage through the column which required only a few seconds. Afterwards the blood behaved perfectly normally with respect to influx and efflux of K. It seemed possible that the precipitate fall of K while the blood was in contact with the resin might be due to the abrupt removal of Ca, not only from plasma, but also from the cellular membrane. As soon as the cell left the resin column, other Ca ions might migrate to the uncovered sites, and thus render the membrane impermeable once again. These inferences are all indirect, and may apply equally to Mg or any other ion which is absorbed strongly by the resin.

Some indication that Ca enjoys a special status with respect to red cell membranes is afforded by experiments of Ponder (32), who placed red cells in isotonic solutions of a variety of cations. After 24 hours at 4°C. the external

cation had replaced much of the intracellular K, as shown in Table I. It is clear that Ca, of all the ions studied, is most effective in entering the cell, and that 2.5 times as much Ca enters as Na. These results do not seem explicable on the basis of passage through the canal, since the charge barrier should be much more effective against Ca than against Na. If, however, the canal were lined with Ca ions, Ca might enter by displacement of other Ca ions along the canal. This interpretation would suggest that Ca plays a more

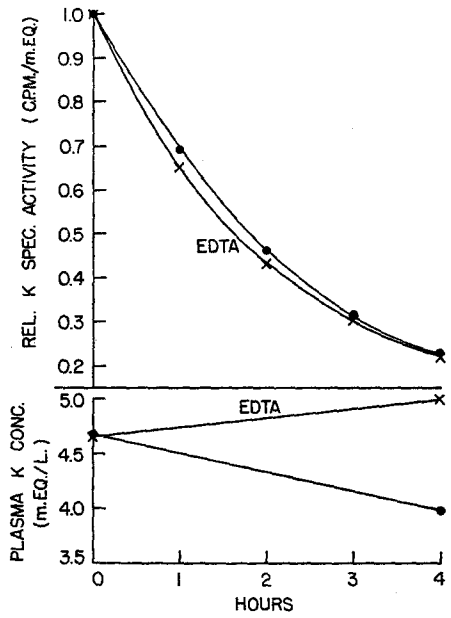

FIGURE 9. The effect of EDTA on K flux in human red cells at 37°C. The upper curve shows the time course of plasma K specific activity. The full circles represent normal controls.

important role in the red cell membrane than do any other of the divalent cations studied.

We have approached the same problem by measuring the effect of ethylenediaminetetraacetic acid[2] (EDTA) on K flux. Fig. 9 shows that EDTA exercises no apparent effect on K influx. On the other hand, the plasma K concentration increases markedly during the course of the experiment, indicating that the cell is losing K, in contradistinction to normal conditions in which the cell takes up K. This conclusion is borne out by numerical examination of the fluxes. K influx in the presence of 25 mM. EDTA is 2.02 mM/liter cell hour, as compared with a control flux of 2.00 mM/liter cell hour; efflux in the presence of EDTA is 2.12 mM/liter cell hour, an increase of 0.32 above

[2] The disodium salt was used.

the control value of 1.80 mM/liter cell hour. Citrate also causes marked loss of intracellular K. Thus it would seem that there is a connection between divalent cations and K flux across the red cell membrane. It is not unlikely that Ca is the ion responsible, but the present state of our knowledge is not sufficient to prove this to be the case. It will remain difficult to unravel the details of the interactions involved until a good analytical method for Ca determination in the red cell membrane is available. Nonetheless, for the present, the concept of a Ca canal seems an attractive speculation.

CONCLUSIONS

We have been concerned with the relationship between red cell membrane structure and the carrier model of K transport, as shown in Fig. 1. Two arguments have been presented which lead to the conclusion that the red cell membrane is penetrated by equivalent pores 3.5 to 4.2 Å in radius. These pores are presumed to contain an assemblage of positive charges which account for the striking difference in the permeability of the red cell membrane to K and Cl. Evidence has been presented to support the notion that the K ion enters the cell by a route different than that taken by the H_2O, as required by the carrier hypothesis. Furthermore, the major pathway for K exit has been shown to be different from that for entrance. It has been suggested that the exit route may be identical with the equivalent pores, whose dimensions are such as to permit passage by hydrated K and Na ions. Finally, evidence has been presented that Ca or another divalent cation is concerned in the exit of K from the red cell. Thus characterization of the red cell membrane in physical terms has led to a structure in general accord with expectations based on the carrier theory of ion transport.

REFERENCES

1. SOLOMON, A. K., *J. Gen. Physiol.*, 1952, **36**, 57.
2. STREETEN, D. H. P., and SOLOMON, A. K., *J. Gen. Physiol.*, 1954, **37**, 643.
3. GOLD, G. L., and SOLOMON, A. K., *J. Gen. Physiol.*, 1955, **38**, 389.
4. TOSTESON, D. C., *Acta Physiol. Scand.*, 1959, **46**, 19.
5. MEYER, K. H., and SIEVERS, J.-F., *Helv. Chim. Acta.*, 1936, **19**, 649.
6. SOLLNER, K., *in* Electrochemistry in Biology and Medicine, (T. Shedlovsky, editor), New York, John Wiley and Sons, 1955, 33.
7. SOLOMON, A. K., GILL, T. J. 3rd, and GOLD, G. L., *J. Gen. Physiol.*, 1956, **40**, 327.
8. KOEFOED-JOHNSEN, V., and USSING, H. H., *Acta Physiol. Scand.*, 1953, **28**, 60.
9. PAPPENHEIMER, J. R., RENKIN, E. M., and BORRERO, L. M., *Am. J. Physiol.*, 1951, **167**, 13.
10. SOLOMON, A. K., *in* Proceedings of the 1st National Biophysics Conference, New Haven, Yale University Press, 1959, 314.

11. PAGANELLI, C. V., and SOLOMON, A. K., *J. Gen. Physiol.*, 1957, **41**, 259.
12. SIDEL, V. W., AND SOLOMON, A. K., *J. Gen. Physiol.*, 1957, **41**, 243.
13. VILLEGAS, R., BARTON, T. C., AND SOLOMON, A. K., *J. Gen. Physiol.*, 1958, **42**, 355.
14. GORIN, M. H., *J. Chem. Physics*, 1939, **7**, 405.
15. STAVERMAN, A. J., *Rec. trav. chim. Pays-bas*, 1951, **70**, 344.
16. DURBIN, R. P., FRANK, H., and SOLOMON, A. K., *J. Gen. Physiol.*, 1956, **39**, 535.
17. DURBIN, R. P., *in* Proceedings of the 1st National Biophysics Conference, New Haven, Yale University Press, 1959, 323.
18. STEIN, W. D., *Nature*, 1958, **181**, 1662.
19. RENKIN, E. M., *J. Gen. Physiol.*, 1954, **38**, 225.
20. PONDER, E., Hemolysis and Related Phenomena, New York, Grune and Stratton Inc., 1948, 82.
21. GLYNN, I. M., *J. Physiol.*, 1957, **136**, 148.
22. LOVELOCK, J. E., *Biochem. J.*, 1955, **60**, 692.
23. JOYCE, C. R. B., MOORE, H., and WEATHERALL, M., *Brit. J. Pharmacol.*, 1954, **9**, 463.
24. PASSOW, H., and TILLMANN, K., *Arch. ges. Physiol.*, 1955, **262**, 23.
25. PASSOW, H., and SCHÜTT, L., *Arch. ges. Physiol.*, 1956, **262**, 193.
26. GRIGARZIK, H., and PASSOW, H., *Arch. ges. Physiol.*, 1958, **267**, 73.
27. VINCENT, P. C., and BLACKBURN, C. R. B., *Australian J. Exp. Biol., and Med. Sc.*, 1958, **36**, 471.
28. WINKLER, K. C., and BUNGENBERG DE JONG, H. G., *Arch. néerl, physiol.*, 1940–41, **25**, 431.
29. WINKLER, K. C., and BUNGENBERG DE JONG, H. G., *Arch. néerl. physiol.*, 1940–41, **25**, 467.
30. WALTERS, D. H., private communication.
31. SOLOMON, A. K., and REES, S. B., communications to the 19th International Physiological Congress, Montreal, 1953, 781.
32. PONDER, E., *J. Gen. Physiol.*, 1953, **36**, 767.