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Permeability of Red Blood Cells to Ions.

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Recent experiments with radioactive isotopes have demonstrated the permeability of red blood cells to cations. The results obtained by different experimenters are, however, not directly comparable with each other or with the permeability of other systems. Permeability and diffusion phenomena ought to be expressed in standard c g s units.[1] With the aid of published measurements on cells of the same species[2] and with certain assumptions, it is possible to evaluate permeabilities in terms of standard units. Following Rashevsky[1] one may define the permeability, h, of a membrane to a given solute (provided the membrane is the limiting factor in the rate of exchange of solute) as

$$h = \frac{Q}{S\ (C_1 - C_2)}$$

where Q is the rate at which the solute crosses the membrane in mols per second, S is the area of the membrane and $C_1 - C_2$ the difference in concentration of the diffusing substance between the two sides of the membrane in mols per cc.

The permeability constant, h, of a membrane is defined on the assumption that the rate of diffusion, Q, is the difference between the rate of exit which is proportional to C_1, and the rate of entry, which is proportional to C_2. In the red cell, however, Q, the net rate of ion change, is zero. The permeability factor, b,[3] measures the fraction of the ions inside the cell which cross the membrane each minute. We may assume, for example, that loss of potassium from the cell is entirely passive and may be regarded as a leak, while the uptake of potassium takes place by another path motivated by an unspecified mechanism. If so, the rate of loss, b, should be proportional to C_1 and not to $C_1 - C_2$. In some experiments, b is measured by the rate of uptake of radioactive potassium; however,

1 Rashevsky, N., and Landahl, H. D., *Cold Spring Harbor Symposia*, 1940, **8**, 9.

2 Ponder, E., *The Mammalian Red Cell and the Properties of Haemolytic Systems*, Berlin, 1934.

3 Dean, R. B., Noonan, T. R., Haege, L., and Fenn, W. O., *J. Gen. Physiol.*, 1941, **24**, 353.

the rate of active uptake equals the rate of passive loss, if there is no net change in total potassium, so conversion of b to permeability units still entails dividing b by C_1. In the case of sodium or anions where there is no total change, we can calculate different constants depending on whether we assume that inward or outward movement is passive. In this paper I have assumed that outward movement is passive. In cases where permeability is measured by a net change of cellular content, of course, $C_1 - C_2$ must be used in the calculation. The concentration C_1 is that of the free potassium in the free water. Since there is no general agreement as to the existence or magnitude of the bound fractions of potassium and water, I have assumed that all the potassium is dissolved in all of the cellular water.

To determine the diffusion constant, D, we have to know the thickness of the cell membrane. For rabbit cells this lies between 110 and 120 Å[4, 5] but it has not been measured for other cells. The effective thickness of the membrane may be as low as 20 Å, the thickness of a monolayer of lipoidal material.[6] For the purposes of this calculation I have taken the larger value. The diffusion constant, D, is given as $D = hd$, where d is the membrane thickness in cm.

TABLE I.
Data Used to Evaluate the Permeability Constants of Various Ions.

	in vivo				*in vitro*
	Rabbit K^+	Human K^+	Dog K^+	Dog Na^+	Ox HCO_3^-
Volume, μ^3; $cm^3 \times 10^{-12}$	60	83	57	57	48
Surface, μ^2; $cm^2 \times 10^{-8}$	110	163	96	96	75
Cv, milimolar; mols, $cm^{-3} \times 10^{-6}$	91	101	8.0	98	11.0*
Ci, milimolar; mols, $cm^{-3} \times 10^{-6}$	144	158	12.4	153	17.2*
d, Å; cm $\times 10^{-8}$	110	(110)	(110)	(110)	(110)
b, $sec^{-1} \times 10^{-5}$	1.1	1.2	3.0	6	500,000
Q, mols, $sec^{-1} \times 10^{-20}$	6.2	11	1.4	34	26,000
h, cm, $sec^{-1} \times 10^{-10}$	3.9	4.1	11	23	200,000
D, cm^2, $sec^{-1} \times 10^{-16}$	4.3	4.5	12	25	220,000

Cv—Concentration of ions in the total cell volume.

Ci—Concentration of ions in the cell water.

*These values for ox cells refer to differences in concentration between cells and plasma.

d—Thickness of cell membrane which has only been measured for rabbit cells. Other cell membranes are assumed to have the same thickness.

b—Fraction of the ions in each cell which leaves each second.

Q—Number of mols of ions in each cell which leaves each second.

h—Is the permeability of the cell membrane to the ions.

D—Permeability constant of the substance forming the membrane to the ions.

In experiments done *in vivo* the cells were centrifuged immediately after the blood was drawn from the animal so that practically all the diffusion occurred while the cells were still in the animal.

4 Waugh, D., and Schmitt, F. O., *Cold Spring Harbor Symposia*, 1940, **8**, 233.

5 Fricke, H., Palmer, E., and Ponder, E., *J. Cell. Comp. Physiol.*, 1939, **13**, 69.

6 Dervichian, D. R., and Macheboeuf, F., *Comptes Rendus*, 1938, **206**, 1511.

Table I shows values of the various factors and calculated values of Q, h, and D for potassium in rabbit, dog, and human cells,[3, 7] and for sodium in dog cells,[8] all *in vivo*. Where measurements of the cells were not available, a reasonable estimate has been made by comparison with other data. These values are indicated by parentheses.

The diffusion of bicarbonate ion out of ox cells *in vitro* has been estimated by analytical methods.[9] These results are not strictly comparable as some of the resistance to diffusion certainly lies in the fluid of the cell. Nevertheless, the data have been evaluated as if all the resistance were in the membrane. The results are probably valid to within an order of magnitude.

The probable errors of the determinations of diffusion rates, b, are of the order of 20%. The other values are probably more accurate, except for the thickness of cells other than the rabbit. Therefore, the constants given here can not be trusted very closely. They should be accurate as to order of magnitude.

For comparison, Table II lists diffusion in bulk materials as well as the diffusion constants reported by Jacobs on cells *in vitro*[10] and converted to c g s units assuming a membrane thickness of 110 Å. It is obvious from Table II that the red cell membrane is extraordinarily impermeable to cations and is about 50,000 times more permeable to anions. If the assumptions underlying these calculations are valid as to order of magnitude, the red cell membrane is less permeable than most dense solids which indicates a very high degree of association between the molecules of its structure. Incidentally, all the equations developed by Jacobs and his school[11] for

TABLE II.
Diffusion constants D in cm^2 sec^{-1}.

KCl in water	1.6×10^{-5}
$ZnSO_4$ in water	1.4×10^{-6}
Starch in water	$8. \times 10^{-7}$
Zn in Cu	2.3×10^{-13}
HCO_3 in ox cells	2.2×10^{-11}
K in rabbit cells	3.9×10^{-16}
Urea in ox cells	2.0×10^{-10}*
Glycerol in ox cells	2.1×10^{-14}*

*These calculations are based on an assumed thickness of 110×10^{-8} cm for the ox red cell membrane.

7 Mullins, L. J., Noonan, T. R., Haege, L., and Fenn, W. O., *Am. J. Physiol.*, 1941, **135**, 93.

8 Cohn, W. E., and Cohn, E. T., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 445.

9 Dirken, M. N. J., and Mook, H. W., *J. Physiol.*, 1931, **73**, 349.

10 Jacobs, M. H., *J. Cell. Comp. Physiol.*, 1934, **4**, 161.

11 Jacobs, M. H., *Cold Spring Harbor Symposia*, 1940, **8**, 30.

the responses of a cell having a membrane impermeable to cations will be approximately true for a cell having a membrane 0.00002 times as permeable to cations as to anions. The rate of loss of potassium from cells where the uptake may have been stopped by a poison is slow enough to be undetectable in the initial lag period found by Davson[12] for the loss of potassium from cells poisoned by respiratory poisons. In his experiments all of the loss must be attributed to a changed permeability of the membrane. The question as to what puts potassium back in the cell against a diffusion gradient remains unanswered. However, the experiments of Danowski[13] and Harris[14] show that metabolism is necessary for the continued uptake of potassium by the cells of stored blood.

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Non-Permeability of Blood Clot to Sulfonamide Drugs in Presence of Increased Temperature.

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Favorable results have been recently reported[1, 2] following the use of induced fever associated with sulfonamide drugs in the treatment of subacute bacterial endocarditis. That sulfonamide drugs do not penetrate fibrin or blood clot to any significant degree has been reported by two separate groups of observers, using different *in vitro* methods.[3, 4] However, a third has found that penetration does take place.[5] It occurred to us that if a high body temperature increased the effectiveness of the sulfonamide drugs in subacute bacterial endocarditis, it could do so only by bringing the drug into contact with the organism within the vegetation; *i. e.*, by increasing the permeability of the vegetation to the drug. To determine this we studied

12 Davson, H., *J. Cell. and Comp. Physiol.*, 1941, **18**, 173.
13 Danowski, T. S., *J. Biol. Chem.*, 1941, **139**, 693.
14 Harris, J. E., *J. Biol. Chem.*, 1941, **141**, 579.
1 Solomon, H. A., *New York State J. Med.*, 1941, **41**, 45.
2 Bierman, W., and Baehr, G., *J. A. M. A.*, 1941, **116**, 292.
3 Duncan, C. N., and Faulkner, J. M., *Am. J. Med. Sci.*, 1940, **200**, 492.
4 Friedman, M., *Arch. Int. Med.*, 1941, **67**, 921.
5 Uhley, M. H., and Katz, L. N., *J. Infect. Dis.*, 1941, **68**, 291.