10705

Permeability of Red Corpuscles of the Dog to Sodium Ion.*

WALDO E. COHN AND ELMA TUFTS COHN. (Introduced by D. M. Greenberg.)

From the Division of Biochemistry, University of California Medical School, Berkeley.

The question of the permeability of mammalian erythrocytes to cations (with the exception of hydrogen ion) has long been a controversial one. The literature in the field has been reviewed by Ponder[1] and Jacobs.[2] Ponder states that, although there has been some evidence since 1891[3] that the red blood cell is permeable to cations, it has generally been assumed that it is not.[4] Such an assumption requires awkward corrections to explain the experimental data. To avoid postulating a cation shift across the red cell membrane, Ponder[1] states that Van Slyke and Cullen,[5] Mellanby and Wood,[6] and Doisy and Eaton[7] have preferred to introduce corrections involving changes in red cell volume. However, Van Slyke and Cullen[5] admit the possibility of a transfer of cations between plasma and corpuscles. For these volume changes, which are ascribed to water shifts, there is no good evidence, the most careful work giving results varying by as much as 100%[8] or even varying in direction.[9] Much of the interpretation is based on hematocrit values which are admittedly unreliable. If there is no water shift, or if it is not of the right magnitude, the results of the investigators mentioned above support the idea of a red blood cell permeable to cations.

Jacobs,[2] on the basis of Donnan ratios, relation of cell volume to osmotic pressure, and volume changes in solutions of varying pH

* Aided by grants from the Division of Natural Sciences of the Rockefeller Foundation and from the Christine Breon Fund of the University of California Medical School.

1 Ponder, E., *The Mammalian Red Cell and the Properties of Hemolytic Systems*, Berlin, 1934, pp. 61, 105-108, 109-114, 128.

2 Jacobs, M. H., *Ergeb. d. Biol.*, 1931, **7**, 1.

3 Hamburger, H. J., *Z. f. Biol.*, 1891, **28**, 405; Hamburger, H. J., and Bubanović, F., *Arch. int. Physiol.*, 1910-11, **10**, 1.

4 Ege, R., *Biochem. Z.*, 1922, **130**, 99; Koeppe, H., *Pflüger's Arch.*, 1897, **67**, 189.

5 Van Slyke, D. D., and Cullen, G. E., *J. Biol. Chem.*, 1917, **30**, 289.

6 Mellanby, J., and Wood, C. C., *J. Physiol.*, 1923, **57**, 113.

7 Doisy, E. A., and Eaton, E. P., *J. Biol. Chem.*, 1921, **47**, 377.

8 Van Slyke, D. D., Wu, H., and McLean, F. C., *J. Biol. Chem.*, 1923, **56**, 765.

9 Warburg, E. J., *Biochem. J.*, 1922, **16**, 153.

(with CO_2), concludes that the red blood cell is impermeable to cations. As Ponder[1] has pointed out, the evidence is inconclusive on all 3 points, and Jacobs himself admits the possibility of alternate explanations.

That a so often investigated subject should still be in such a controversial state is due principally to the indirectness of the previously available methods of study. The most direct experiments were performed by adding salts to blood *in vitro* and measuring the changes in cation concentration and in volume of whole blood, plasma and cells.[10] The time for any permeation to occur was thus limited to a relatively few minutes and the results depended upon the analysis of very small differences in the cation content and volume of the cells. The more indirect methods and experiments with cells in saline or under other abnormal conditions are subject to criticism.[1]

The discovery of the artificial radioactive isotopes of sodium and potassium has opened up an entirely new method of approach, making it possible to study the problem *in vivo* by simple, direct methods.[11] The artificial radioactive isotope of sodium (Na^{24}) has the same chemical behavior as the naturally occurring one (Na^{23}), yet may be detected and measured by its decomposition in which it emits electrons and gamma radiation.

We are investigating the subject of the permeability of mammalian erythrocytes to cations with this method and report herewith the results of experiments with radioactive sodium in the dog.

Radioactive sodium,† in 10-25 ml isotonic saline, was injected intravenously into normal dogs. At various periods of time (captioned *"in vivo"* in the table), blood samples were drawn into heparin and centrifuged after varying periods of time (*"in vitro"* in the table) for 10 minutes at about 2400 r.p.m. Except where it is stated that the sample was drawn under oil, no precautions were taken against loss of CO_2. After centrifugation, the plasma was withdrawn as completely as possible with a pipette and the cells were mixed with a large volume of isotonic sucrose (equal to 1.12% NaCl) and centrifuged. This was repeated twice to remove all adherent plasma. No evidence of loss of sodium from the cells was

[10] Eisenman, A. J., Mackenzie, L. B., and Peters, J. P., *J. Biol. Chem.*, 1936, **116**, 33; Wakeman, A. M., Eisenman, A. J., and Peters, J. P., *J. Biol. Chem.*, 1927, **73**, 567.

[11] Hevesy, G., *Enzymologia*, 1938, **5**, 138; Lawrence, J. H., *Handbook of Physical Therapy*, Am. Med. Assn., 1938.

† We are indebted to Professor E. O. Lawrence and the staff of the Radiation Laboratory of the University of California for supplying us with the radioactive sodium used in these experiments.

TABLE I.

Exp.	Sample	Time (minutes) *In Vivo*	Time (minutes) *In Vitro*	Time (minutes) Total	Log t	Hematocrit (H)	$\frac{Na^{24}/ml\ Red\ Corpuscles}{Na^{24}/ml\ Plasma} \times 100$
4	1A	7	8	15	1.176	44	9.4
	2	32	13	45	1.653	44	12.6
	1B	7	143	150	2.176	51	25
5	1A	7	3	10	1.000	47	5.5
	2A	27	4	31	1.491	51	10
	3A	45	2	47	1.672	51	11
	4	397	3	400	2.602	47	44
	5	1530	5	1535	3.186	47	65
	1B	7	94	101	2.004	45	22.4
	2B	27	74	101	2.004	48	23
	3B	45	56	101	2.004	52	20
8	1	5	6	11	1.041	54	4.5
	2	5	32	37	1.568	53	7.8
	3	5	61	66	1.820	54	14
	4	5	182	187	2.272	53	32
11	1A	5	15 (oil)	20	1.301	52	4.8
	1B	5	134 (oil)	139	2.143	52	23
	1C	5	279 (oil)	284	2.453	51	32
	2	135	4	139	2.143	51	20
	3	280	4	284	2.453	50	27
13	1	840	10	850	2.929	56	56
	2	1020	10	1030	3.013	56	56

Time *in vivo* = time between injection of Na^{24} and withdrawal of blood sample.
Time *in vitro* = time between withdrawal and centrifugation.

noted, and in several cases measurements of the wash solutions and hematocrits showed a Na^{24} content equal to the plasma left behind.

The relative amount of Na^{24} in plasma was measured directly, after evaporating an aliquot in a 10 ml Coors ashing capsule, by means of a Lauritzen electroscope. The relative amounts of Na^{24} in whole blood and cells were measured on trichloracetic filtrates. Corrections were made for the volume of the protein precipitate in each case. The radioactivity of each sample is expressed in arbitrary units and is directly proportional to the Na^{24} content.

The data and results are shown in Table I. The hematocrit values were calculated from the Na^{24} content of whole blood, plasma, and cells and agreed with those found (from graduated centrifuge tubes) within 5 volume %.

In Figure 1 the ratios of red corpuscle Na^{24} to plasma Na^{24} have been plotted against the logarithm of the total time which elapsed

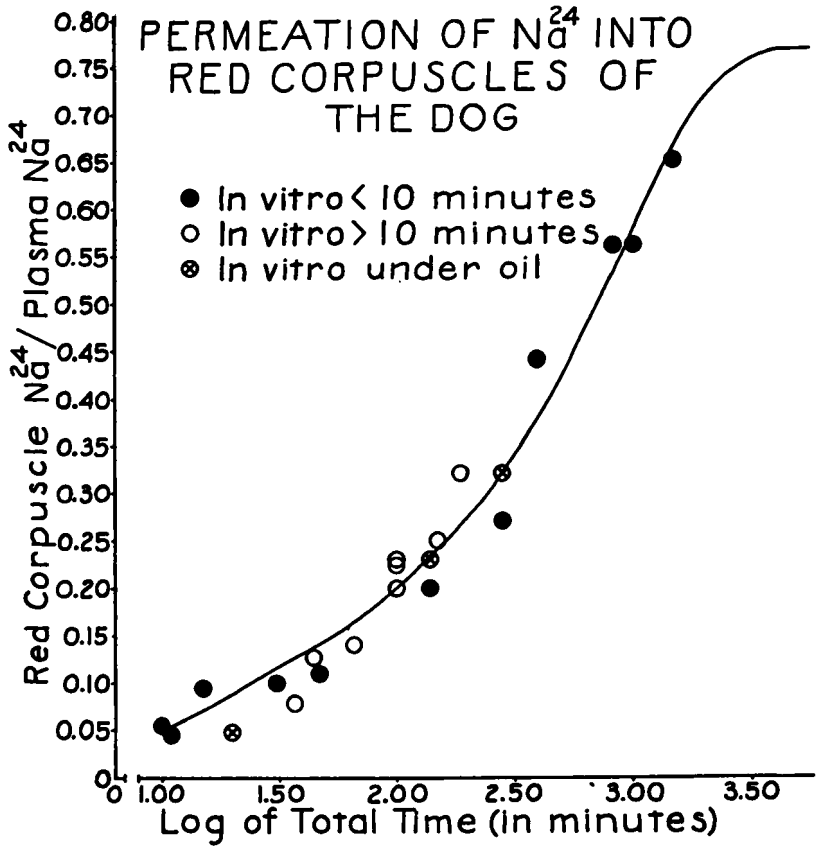

FIG. 1.

from the injection of Na^{24} to the centrifugation of the blood samples. The points represent the experimental data. The solid curve has been calculated by assuming that a simple equilibrium exists between corpuscle and plasma sodium, and that the rate at which Na^{24} enters the corpuscle from the plasma is proportional to the difference in their respective concentrations, allowing for the normal difference in sodium content.

The following conclusions may be drawn from these results: first, that the red corpuscles of the dog are permeable to sodium ion; second, that the rate of permeation by sodium ion is nearly the same with blood *in vitro* as *in vivo;* third, that the permeation appears to be of a simple equilibrium nature.

We wish to express our gratitude to Professor D. M. Greenberg for his advice and assistance in these experiments, and to Mr. A. J. Glazko for technical aid.

10706

Blood Sugar in Cats with Diabetes Insipidus Before and After Adrenalectomy.

W. R. INGRAM AND C. A. WINTER.

From the Departments of Anatomy and Physiology, State University of Iowa.

Cats with experimental diabetes insipidus produced by interruption of the nervous connections of the posterior pituitary show rather unusual effects after bilateral adrenalectomy.[1, 2, 3] Whereas ordinary adrenalectomized cats show striking diminution in the serum sodium and chloride levels, adrenalectomized d.i. cats have normal serum concentrations of these substances even when *in extremis*. There is a more or less marked elevation of blood potassium, however, similar to that occurring in ordinary animals. The survival time after adrenalectomy is reduced from an average of 8 days for ordinary cats to an average of 3 for d.i. cats. A negative water balance is set up in both cases, the d.i. cats losing water the more rapidly. The total loss of water is not a lethal one; however, it has been suggested that the rapid external loss plus shifts in fluid within

[1] Winter, C. A., Gross, E. G., and Ingram, W. R., *J. Exp. Med.*, 1938, **67**, 251.

[2] Ingram, W. R., Winter, C. A., and Gross, E. G., *Am. J. Physiol.*, 1938, **122**, 143.

[3] Winter, C. A., Ingram, W. R., and Gross, E. G., 1939, in press.