XXXV. PERMEABILITY OF VITAL MEMBRANES.

THE RED BLOOD CORPUSCLE.

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THE red blood corpuscle possesses a remarkable property of retaining potassium as 99 % of its cations [Kramer and Tisdall, 1922], whilst the proportion of potassium in the surrounding serum is extremely low; at the same time the exchange of oxygen and some other substances is practically unhindered. In the study of vital membranes it was thought that the red blood corpuscle, being so easily obtainable, might afford indications of the passage of therapeutic substances and foodstuffs through other vital membranes. A considerable amount of work has already been done on this subject. Höber [1912] found that the property of permeability of the corpuscular envelope to ammonium was altered by various conditions, of special interest being his work with toxic substances. Noguchi [1905] showed that cobra venom under certain conditions made the corpuscles absolutely impermeable even to distilled water. Winokuroff [1929] found that partially haemolysed corpuscles, reverted by saline, had lost their selective permeability to anions and cations.

Nicloux and Yovanovitch [1925] determined the distribution of ethylene to be about 75 % in the corpuscles and 25 % in the plasma.

METHODS.

In the tests which follow, the concentrations of substances in solution were determined before and after contact with a known volume of washed corpuscles. We first of all found that the erythrocyte showed absolute impermeability to sulphates and ready permeability to bromides, and by utilising a solution of known strength of bromide and sulphate *plus* substance to be tested, the errors consequent upon large volumes of quasi-solid material in the mixtures could be eliminated. From a theoretical point of view one need not even know the initial volume of blood corpuscles, the figures being obtainable by mathematical calculation from the proportions of sulphate and bromide before and after the test.

Sheep's blood corpuscles were washed several times by centrifuging with saline and the volume of corpuscles, after centrifuging for half an hour, was measured. A solution of the substance to be tested, adjusted to p_H 7.4, was added in the proportion of 1 of solution to 3 of corpuscles. After thorough mixing during half an hour the mixture was again centrifuged and the supernatant solution analysed.

By the use of masses of raw egg-white suspended by wire hooks in a solution, we were able to investigate selective permeability of albuminous masses without any intervening lipid envelope. Ten minutes in a solution containing equal proportions of bromide and sulphate resulted in penetration of five times as much bromide as sulphate. The difference in velocities of the ions in water being insignificant there was thus a real selective permeability.

In view of the highly albuminous nature of the red blood corpuscle this property may be of great importance in addition to the lipid envelope. Both factors taken together constitute an ideal condition resembling a vital membrane with its adjacent protoplasm elsewhere in the body.

RESULTS.

(1) INORGANIC.

A. Cations.

(a) *Potassium*. It may be taken without further experimental proof that the erythrocyte is impermeable to potassium.

(b) *Ammonium*. Determination of NH_3 was made using three salts—chloride, bromide and sulphate. In each case ammonium was found to penetrate readily, attaining equal proportions with the solute in a short time (Table I, Exps. 1, 2, 3).

(c) *Copper*. Copper tartrate was used; contact of solution with corpuscles for over 1 hour resulted in only slight diminution of copper, the amount not exceeding the probable surface absorption (Table I, Exp. 4).

(d) *Calcium*. $CaCl_2$ used; contact for 1 hour resulted in practically no passage of calcium. (In this experiment it was necessary to lower the p_H to 7.2 (Table I, Exp. 5).) Leiboff [1930] found that calcium is entirely absent from the corpuscles of human blood.

B. Anions.

(a) *Sulphate*. Used in the form of salts of sodium, potassium and ammonium. No permeation of the corpuscle occurred (Table I, Exp. 6 and others).

(b) *Chloride*. Used in the form of sodium and ammonium salts. The corpuscles were readily permeable to chloride ion (Table I, Exps. 7 and 8).

(c) *Bromide*. Employed as sodium and ammonium salts. With ammonium bromide it was necessary to reduce the time of contact with the blood to avoid haemolysis, and to make the p_H of the solution 6.8 (2 % disodium hydrogen

phosphate, p_H 6.0, did not cause haemolysis of the same specimen of blood) (Table I, Exps. 9a, 9b, 9c).

(d) *Dichromate*. As ammonium and potassium salts. There was a definite penetration of the corpuscle, which became brown owing to the formation of

Table I. *Permeability of red blood corpuscles for inorganic ions.*

(Single salts.)					
Experi- ment	Ion	Salt	Initial con- centration of salt %	Concentration of ion in corpuscles % Concentration of ion in liquid %	Permeability, i.e. (quotient $\times 100$)
1	NH ₄	NH ₄ Cl	4.0	$\frac{0.42}{0.42}$	100
2	NH ₄	NH ₄ Br	4.0	$\frac{0.36}{0.36}$	100
3	NH ₄	(NH ₄) ₂ SO ₄	2.0	$\frac{0.18}{0.19}$	95
4	Cu	Cu tartrate	3.0 Cu	$\frac{0.4}{2.6}$	15
5	Ca	CaCl ₂	31 mg. Ca per cc.	$\frac{0.6}{19.4}$ mg. per cc.	3
6	SO ₄	Na ₂ SO ₄ , 10H ₂ O	2.81	$\frac{0.04}{0.77}$	5
7	Cl	NaCl	1.5	$\frac{0.31}{0.32}$	99
8	Cl	NH ₄ Cl	4.0	$\frac{0.46}{1.5}$	30
9a	Br	NaBr	1.5	$\frac{0.58}{0.58}$	100
9b	Br	NaBr	2.5	$\frac{0.66}{0.66}$	100
9c	Br	NH ₄ Br	4.0	$\frac{1.06}{1.06}$	100
10a	Cr ₂ O ₇	K ₂ Cr ₂ O ₇	5.0	$\frac{1.23}{1.19}$	100
10b*	Cr ₂ O ₇	K ₂ Cr ₂ O ₇	2.75	$\frac{0.59}{0.84}$	70
11	Cr ₂ O ₇	(NH ₄) ₂ Cr ₂ O ₇	0.42	$\frac{0.104}{0.139}$	75
12	Fe(CN) ₆ †	K ₄ Fe(CN) ₆	5.0	$\frac{0.20}{2.8}$	7
13a	(COO) ₂	(COOK) ₂	4.0	$\frac{0.29}{1.33}$	22
13b	(COO) ₂	(COOK) ₂	13.0	$\frac{\dagger 1.76}{3.8}$	46

* N/20 dichromate added = 55.2 cc. N/20 dichromate removed in supernatant liquid = 26.4 cc. Dichromate removed from corpuscles by washing 4 times with 5 cc. of saline = 26.5 cc. Total dichromate recovered = 52.9 cc.

In this Exp. the corpuscles, after exposure to the dichromate, contained only 10 % of oxyhaemoglobin, the remainder being converted to methaemoglobin.

† Expressed as oxalic acid.

methaemoglobin within an apparently intact membrane. After centrifuging it was found that, by washing with saline, the dichromate which had penetrated the corpuscles could be recovered (Table I, Exps. 10 and 11).

(e) *Ferrocyanide*. Used as potassium ferrocyanide. No penetration occurred (Table I, Exp. 12).

(f) *Oxalate*. Used in the form of a 4 % solution of potassium oxalate; also in stronger concentration of about 13 % after neutralisation by titrating with oxalic acid solution to p_H 7.2. A definite decrease in the concentration of oxalate of surrounding solute was observed (Table I, Exp. 13) indicating some penetration.

(g) *Ferricyanide*. Used as potassium ferricyanide. No penetration occurred.

The test with ferricyanide solution thus showed marked contrast with the dichromate in that no change in the constitution of the haemoglobin occurred whilst protected by the corpuscular envelope. The oxygen capacity of the blood corpuscles (determined according to Woodhouse and Pickworth [1930]) was unaffected even by contact with 8 % potassium ferricyanide. Similar analyses of blood corpuscles after contact with $N/2$ potassium dichromate showed that 90 % of the oxyhaemoglobin was converted to methaemoglobin.

Experiments using $N/20$ potassium permanganate and varying concentrations of potassium mercuric iodide failed, owing to the destructive action on the corpuscles, which were quickly haemolysed, the haemoglobin being converted to methaemoglobin.

(h) *Bromide and sulphate*. Washed corpuscles were mixed with 1.5 % sodium bromide + 2.8 % sodium sulphate. After a suitable period of contact and centrifuging, analysis showed that the bromide had penetrated readily and the sulphate not at all (Table II B, Exp. 1).

(i) *Bromide, sulphate and dichromate*. A mixture containing 3 % sodium sulphate, 2 % sodium bromide and 2.5 % potassium dichromate was left in contact with the corpuscles for half an hour.

The penetrations of the ions in this experiment were equivalent to those which took place in absence of the other salts. Although the haemoglobin was largely converted to methaemoglobin, the bromide penetrated readily and the sulphate not at all. The selective properties of the membrane were not affected even although complete alteration in the chemical constitution of the protein within the corpuscle occurred (Table II B, Exp. 2).

(2) ORGANIC.

Dyes.

The distribution of a number of water-soluble dyes between corpuscles and the liquid phase was studied by estimating the amount of colour remaining in solution before and after contact with the washed corpuscles for 30 minutes. In most cases the Lovibond tintometer was employed but in a

few cases comparison was made in a Klett colorimeter. Various concentrations of dye were tried.

The dyes employed fell into 4 groups.

(1) Impermeable (permeability quotient 0-2.6)—trypan red, trypan blue, naphthol green, eosin, erythrosin, phenol red, acriflavine, Congo red (0.05 %).

(2) Semi-permeable (permeability quotient 5-10)—naphthol black, orcein, safranine, carmine, neutral red.

(3) Permeable (permeability quotient 33-100)—acid fuchsin, pyronin, rosolic acid, toluidine blue, Bismarck brown, nigrosine, Congo red (0.5 %).

Table II. *Permeability of red blood corpuscles.*

Experi- ment	Ion tested	Compound	Initial con- centration %	Concentration in corpuscles % Concentration in liquid %	Perme- ability (i.e. quotient × 100)
(A) Organic compounds.					
1a	—	Urea in isotonic saline	10	$\frac{3.48}{5.5}$	63
1b	—	„	5	$\frac{1.65}{2.62}$	63
1c	—	„	1	$\frac{0.30}{0.50}$	60
1d	—	„	1	$\frac{0.20}{0.33}$	61
2a	—	Glucose	5	$\frac{1.08}{2.85}$	38
2b	—	„	2	$\frac{0.45}{1.10}$	41
(B) Inorganic mixtures.					
1	Br	Sodium bromide 1.5 % +	0.98	$\frac{0.48}{0.49}$	100
	SO ₄	Na ₂ SO ₄ , 10H ₂ O 2.8 %	0.85	$\frac{0.0077}{0.68}$	1.1
2	Br	Sodium bromide 2.0 % +	1.30	$\frac{0.606}{0.56}$	100
	SO ₄	Na ₂ SO ₄ , 10H ₂ O 3 % +	0.89	$\frac{0.030}{0.63}$	5
	Cr ₂ O ₇	Potassium di- chromate 2.5 %	1.69	$\frac{1.12}{1.32}$	85
(C) Mixed organic and inorganic substances.					
1	SO ₄	Na ₂ SO ₄ , 10H ₂ O	3.0	$\frac{0.0465}{0.88}$	5
	Urea	Urea	1.0	$\frac{0.29}{0.50}$	58
2	Glucose	Glucose (pure)	2.0	$\frac{0.44}{1.14}$	39
	Bromide	Sodium bromide	2.0	$\frac{0.52}{0.54}$	100
	Sulphate	Na ₂ SO ₄ , 10H ₂ O	3.0	$\frac{0.040}{0.83}$	5

(4) Indeterminate (adsorption or chemical combination making estimation of permeability inaccurate)—china blue, wasser blau, Nile blue sulphate, methylene blue, janus green, gentian violet, thionine blue, victoria blue, brasilin, brilliant green, dahlia, carminic acid, Biebrich scarlet.

Organic compounds of physiological importance.

Urea. It was found that over a wide range of concentrations of urea the final ratio of urea in corpuscles to fluid was 1.0/1.6. The initial concentrations used and the final values are given in Table II A.

Urea and sulphate. A solution of 1 % urea and 3 % sodium sulphate was employed. It was found that the urea penetrated easily and the sulphate radical remained entirely outside the corpuscle (Table II c, Exp. 1). (Lundsgaard and Holbøll [1926] found that fructose, galactose, acetone and urea all distributed themselves between plasma and corpuscles in the ratio of 1.4/1. Duval [1923] also found the blood corpuscles to be easily permeable to urea and dimethylurea.)

Glucose. Solutions of 5 % aqueous glucose and 2 % glucose in saline were employed. Contact with corpuscles caused a marked decrease in concentration of the glucose. (The glycolytic action of the blood was controlled by another experiment with the addition of sodium fluoride.) An easy penetration of the corpuscles by glucose occurred (see Table II A, Exp. 2).

Glucose + bromide + sulphate. Glucose and bromide each readily entered the corpuscles, being unaffected by the other constituents of the mixtures. Sulphate again remained entirely outside the corpuscles (see Table II c, Exp. 2).

PHYSICO-CHEMICAL ALTERATIONS IN THE CONDITION OF
LIPOID ENVELOPE OF CORPUSCLE.

It has been demonstrated (Table II B, Exp. 2) that conversion of haemoglobin to methaemoglobin by dichromate did not alter the permeability of the cells to the other substances used. It is therefore of practical importance to know the effect of certain substances of physiological or therapeutic interest which presumably act on the envelope.

The following were substances employed: (1) formaldehyde, (2) saponin, (3) veronal, (4) streptococcus toxin.

(1) In concentration of 1–2 % neutralised formaldehyde saline caused the washed corpuscles to become a gelatinous, compact mass after exposing for 3 minutes. Haemolysis was not observed. With 0.4 % solution of formaldehyde there were no changes observed in the structure and no apparent difference from normal corpuscles in the relative permeabilities of bromide and sulphate.

(2) The quantity of saponin added was 0.5 cc. of 0.1 % solution in saline to 5 cc. of washed corpuscles, contact being allowed for 30 minutes. The corpuscles were then again washed with saline and tested with bromide-sulphate

mixture. No measurable change was found to have occurred in the permeability of bromide or sulphate.

(3) Veronal¹ and (4) streptococcus toxin (non-haemolytic) similarly gave no appreciable changes in the permeability in the concentration used.

SEMI-PERMEABILITY OF PROTEIN GEL (RAW EGG-ALBUMIN).

20 cc. egg-white were placed in a collodion bag which was suspended in 20 cc. of a mixture of sulphate 1.4 % and bromide 0.75 %. After 20 hours, the liquid was analysed with the following result: sulphate 0.73 %, bromide 0.42 %. Thus both sulphate and bromide penetrated to very similar extents. Since this test gave no indication of the relative speeds with which the equilibrium with respect to the two ions was attained, and as the collodion membrane introduced a factor of uncertainty, experiments were devised to avoid this by using masses of raw egg-white in direct contact with the solution.

Direct exposure of egg-white to salt solutions.

Small compact masses of egg-white were suspended in tubes containing sulphate and bromide. After intervals of 10–60 minutes these were withdrawn, the supernatant liquid removed by blotting-paper and the content of bromide and sulphate determined in the albuminous mass.

The weight of the specimen being determined, the albumin was coagulated by boiling with a few cc. of water acidified with dilute acetic acid, the clear liquid filtered, made up to 10 cc. and the bromide and sulphate estimated in the usual manner. A control analysis was made by adding 0.5 g. of egg-white to 0.5 cc. of the solution of salts and carrying out the analyses as above.

The results are given in Table III.

Table III. *Permeation of raw egg-albumin.*

Time in minutes	Sulphate (per g. of albumin) mg.	Bromide (per g. of albumin) mg.	Permeability ratio:	
			Bromide Sulphate	Bromide Sulphate
10	1.8	3.61	2.0	5.0
10	2.9	3.36	1.2	3.0
12	3.2	5.28	1.7	4.1
15	11.4	8.02	0.7	1.8
15	11.2	8.10	0.73	1.8
60	15.4	8.57	0.56	1.4
60	15.3	8.28	0.54	1.4
Control	24.3	9.8	0.40	1.0
Original solution	1 cc. = 10 mg. NaBr = 25 mg. Na ₂ SO ₄ , 10H ₂ O			

Thus the bromide in 10 minutes had penetrated 5 times as much as the sulphate. After 1 hour, 85 % of the bromide, but only 62 % of the sulphate

¹ Fabre and Fredet [1925] after intravenous injections of veronal found the ratio

$$\frac{\text{veronal in corpuscles}}{\text{veronal in serum}} = 1.88$$

had passed into the albumin. The difference is too great to be accounted for entirely by the relative mobilities of the individual ions and shows that the protein content of the corpuscle envelope controls to a marked extent the entry or exclusion of ions.

Differential permeation through egg-white.

A glass cell $3'' \times 1\frac{1}{2}''$ was divided into three compartments by two parchment membranes fixed to the sides by wax so that direct flow of liquid from section to section could not occur. 15 cc. of egg-white were placed in the central portion and bromide + sulphate solution was placed in one end, section *A*, and distilled water in the other, *B*.

After 12 hours, bromide had passed into the central compartment but none appeared in section *B*. After 30 hours, bromide appeared in compartment *B* and the following ratios were found:

$$\frac{\text{Bromide in } B}{\text{Bromide in } A} = \frac{1}{2.5},$$

$$\frac{\text{Sulphate in } B}{\text{Sulphate in } A} = \frac{1}{16}.$$

Thus bromide had preferentially migrated, confirming the results obtained in the preceding experiments.

SUMMARY.

The permeability of the red corpuscle to various inorganic ions, dyes and organic compounds of physiological importance has been studied.

By analogy with the permeability of raw egg-white it is shown that the protein as well as the lipid envelope exerts a selective permeability to salts.

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