CLVIII. THE CATAPHORESIS OF MAMMALIAN ERYTHROCYTES.

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THE surface charge of the corpuscular elements of the blood is determined by the nature of the corpuscle membrane. It is therefore obvious that investigation of this surface charge is of great service in the elucidation of the constitution and behaviour of the cell surface. Many workers have attacked this problem but in the majority of cases the results have been only of a semi-quantitative nature or have been obtained under ill-defined conditions. Thus Kozawa [1914] recorded that the erythrocytes of various animals could be brought to an isoelectric condition at a reaction characteristic for each animal, the p_H decreasing in the series: rabbit > guinea-pig > cat, man > dog > pig. Kosaka and Seki [1921] found that when suspended in physiological saline the following series in order of increasing velocity of electrophoretic migration was observed: rabbit < pig < guinea-pig < man < mouse < rat, cat, dog. This order was reversed on suspension in 9.5 % sucrose.

That the cataphoretic velocity of the erythrocytes was dependent on the reaction of the surrounding medium was first postulated by Kozawa [1914] and confirmed by Eggerth [1924] and by Netter [1925]. These workers observed that degrees of acidity or of salt concentration which were comparatively far removed from the physiological range resulted in a changing velocity indicating an unstable condition due to damage of the cell membrane.

Bernardi [1926] recorded the following series for the rates of cataphoretic migration: rabbit < cat < guinea-pig < man < pig, a series which was not in entire agreement with that of Kosaka and Seki. The whole question has been recently placed on a sound basis by the excellent work of Freundlich and Abramson [1927]. These workers established the fact that the velocity of erythrocytes and leucocytes of different adult horses when suspended in serum or plasma did not vary greatly, although the velocity of the red cell was markedly different from that of the white cell.

Abramson [1929] has carried out an extensive investigation of the cataphoretic velocity of erythrocytes and this investigation will be referred to in detail owing to its importance as the first research of this type performed under standard conditions and also to the fact that the experiments about to be described were based on his technique.

Abramson made use of the micro-cell of Northrop and Kunitz [1925]. One drop of the freshly drawn blood was added to 25–50 ml. of $M/15$ phosphate buffer at p_H 7.35 ± 0.03 . Measurements were made at room temperatures (22 – 27°) and corrected to 25° on the assumption that the temperature coefficient of the cataphoresis of the red cell in the medium employed was 0.02 per $^\circ C$. No significant change over a period of 24 hours occurred in the velocity of the red cells investigated when suspended in the above phosphate buffer. The

following values were obtained for the cataphoretic velocities of mammalian erythrocytes, expressed as $\mu/\text{sec.}$, volt/cm. and corrected to 25° . In every instance the cell was negatively charged and hence the movement was towards the anode: rabbit, 0.55; sloth, 0.97; pig, 0.98; opossum, 1.07; guinea-pig, 1.11; man, 1.31; monkey, 1.33; mouse, 1.40; cat, 1.39; rat, 1.45; dog, 1.65. The order, but not the absolute values, was the same in buffered isotonic glucose. No relationship between zoological species and cataphoretic velocity was demonstrable. With regard to human erythrocytes no marked difference in velocity could be detected following the incidence of pregnancy or of severe anaemia, although in the latter instance distinct changes in the morphology of the cells were present.

Perhaps the most important observation during this work was that the red cells, unlike the leucocytes, appeared to be incapable of any surface union with the proteins of the corresponding plasma. Thus human and rabbit erythrocytes exhibited the same difference in velocity when compared in rabbit or human serum. Again, whilst cholesterol crystals and quartz particles adsorbed gelatin from dilute solution in the phosphate buffer, the erythrocytes, even after 24 hours' contact with the gelatin solution, retained their original velocity.

In view of the importance of Abramson's results it was decided to endeavour to determine the velocities of various mammalian erythrocytes with the aid of the macro-apparatus described elsewhere [Prideaux and Howitt, 1930].

EXPERIMENTAL.

A stock solution of $M/15$ phosphate buffer was prepared from A.R. KH_2PO_4 and Na_2HPO_4 . The reaction was determined by means of the hydrogen electrode and adjusted to $p_{\text{H}} 7.37 \pm 0.02$. The apparatus was used with secondary electrodes as described in the experiments on insulin and serum-globulin [Howitt and Prideaux, 1932, 1, 2]. The lower part of the U-tube was filled with a suspension of whole blood in the buffer (approximately 0.5 ml. of blood added to 30 ml. of buffer) whilst the upper part was filled in the usual way with buffer alone. Following levelling and opening of the main taps, the tap at the bottom of the U-tube was carefully opened so that the two surfaces of separation slowly sank until they reached levels approximately agreeing with the lower 10 mm. graduation of each limb, at which point the lower tap was closed. With care this adjustment of the levels could be performed with an excellent retention of the surface boundaries. The erythrocytes were thus acting as self-indicators of their cataphoretic velocities. The cataphoresis was then carried out at room temperature, an E.M.F. of about 100 volts being applied at the secondary electrodes in order to balance the potential due to the mains applied at the primary electrodes. The samples of blood were collected not longer than 12 hours before the actual experiment.

In calculating the actual velocity from the "up" and "down" readings consideration must be given to the effect of gravity, *i.e.* to the velocity of sedimentation of the erythrocytes. It may be noted that this correction was not needed in the micro-method used by Abramson and Freundlich, since in these experiments the particles were viewed from above and only the horizontal component of velocity, *i.e.* that due to the electric field, was actually measured.

In the case of the macro-apparatus described above this sedimentation velocity must be taken into consideration and this is achieved by a simple calculation on the "up" and "down" velocities. Thus, supposing the true *mean* velocity of the corpuscle is x and the rate of sedimentation is y , the "up"

velocity would actually be observed as $x-y$ and the "down" velocity as $x+y$. From this it follows that the true mean velocity is given by half the algebraic sum of the observed "up" and "down" velocities. Thus as an example:

- (a) Velocity in + electrode limb = 6 mm./hr. down.
 (b) Velocity in - electrode limb = 22 mm./hr. down.

The value for (a) must obviously be expressed as -6 for in this case y is greater than x . Hence the true mean velocity is $(22-6)/2 = 8$ mm./hr.

Incidentally the sedimentation velocity in this particular example is 14 mm./hr. or nearly twice that of the cataphoretic migration.

In converting the true mean velocity into a value expressed in $\mu/\text{sec.}$, volt/cm. it was considered justifiable on the ground of the approximate equality of conductivity of suspension and plain buffer to modify the Krut equation (i) to the simplified form (ii)

$$(i) \quad v = \frac{w_1 h_1 + w_2 h_2}{tE};$$

$$(ii) \quad v = \frac{v' (h_1 + h_2)}{tE};$$

where v' is the true mean velocity.

Now $h_1 + h_2 = 274$ and hence the equation becomes

$$v = \frac{274 \times v'}{tE},$$

where t and E have their usual significance.

Thus, completing the example given above,

$$v' = 8 \text{ mm. } t = 3600 \text{ secs. } E = 110 \text{ volts.}$$

$$v = \frac{274 \times 0.8}{3600 \times 110} \times 1000 = 0.53 \mu/\text{sec.}, \text{ volt/cm.}$$

The cataphoretic velocities of the erythrocytes of the following animals were determined: rat, rabbit, mouse, guinea-pig.

In the case of mouse-blood a determination was made on a suspension in the presence of insulin (0.02 %) and also on a suspension of blood from mice suffering from hypoglycaemic convulsions induced by administration of insulin. With each of these two determinations three experiments were carried out (at 37°) using suspensions of blood alone and of blood in the presence of glucose (0.1 %) and of glucose (0.1 %) together with insulin (0.02 %) respectively.

A further determination was made on blood from mice which had been used for the testing of complex arsenic derivatives and which, in the normal course of events, would have died within a few days from arsenical poisoning.

These results, expressed in $\mu/\text{sec.}$, volt/cm., are given in Table I.

On the whole the agreement between the results yielded by the micro- and macro-methods is excellent. An obvious advantage of the macro-method is that the observation of a large number of cells is achieved and hence the average behaviour of a single cell is obtained with a greater accuracy than is derived from the observation of a solitary corpuscle. There appears to be a tendency for the macro-method to yield slightly higher values.

It will be observed that the value given by Abramson for the mouse (1.35 at 25°) is equivalent to one of 1.74 when corrected to 37° whilst the corresponding values obtained by the macro-method are 1.42 and 1.86 respectively. Actual determination at 37° gives a value of approximately 1.9 and hence it appears probable that the value of 1.42 (at 25°) is more acceptable than that of 1.35. In addition the fact that a temperature coefficient of approximately

Table I. *Cataphoretic velocities of some mammalian erythrocytes in M/15 phosphate buffer at p_H 7.37 ± 0.02.*

I. <i>Rat.</i> (Abramson—1.45 at 25°.)				
Animal No.	<i>v</i>	Temp.	<i>v</i> (25°)	
1	1.15	16	1.43	
2	1.34	17.5	1.59	
3	1.02	16	1.28	
4	1.22	14.5	1.56	
5	1.09	14.5	1.41	
		Average	1.45	

II. <i>Rabbit.</i> (Abramson—0.55 at 25°.)				
Animal No.	<i>v</i>	Temp.	<i>v</i> (25°)	
1	0.53	15	0.68	
2	0.52	18	0.61	
	0.42	18	0.49	
3	0.49	15	0.62	
	0.49	15	0.62	
		Average	0.60	

III. <i>Mouse.</i> (Abramson—1.35 at 25°.)				
Animal No.	<i>v</i>	Temp.	<i>v</i> (25°)	<i>v</i> (37°)
1	1.16	15	1.44	1.89
2	1.12	15	1.40	1.82
3	1.94	37	—	1.94
		Average	1.42	

“Arsenical mouse”				
Animal No.	<i>v</i>	Temp.	<i>v</i> (25°)	<i>v</i> (37°)
1	1.16	15	1.44	1.89
2	1.16	15	1.44	1.89
	1.14	16	1.42	1.86
		Average	1.43	

Normal mouse (insulin-glucose experiment). Determinations at 37°.

Blood alone	...	...	...	...	1.84
Blood—glucose	...	...	...	...	1.92
Blood—glucose—insulin	...	...	...	...	1.95

Insulinised mouse (insulin-glucose experiment). Determinations at 37°.

Blood alone	...	...	...	...	1.92
Blood—glucose	...	...	...	...	1.98
Blood—glucose—insulin	...	...	...	...	1.77

IV. <i>Guinea-pig.</i> (Abramson—1.11 at 25°.)				
Animal No.	<i>v</i>	Temp.	<i>v</i> (25°)	
1	0.96	18	1.16	

Summarising the average values we obtain the following results (corrected to 25°) for animals, the corresponding values of Abramson being given in parenthesis for comparison figures indicate the velocity of the corpuscles towards the anode.

Rat ...	...	...	...	...	1.45 (1.45)
Rabbit	...	...	...	...	0.60 (0.55)
Mouse	...	...	...	...	1.42 (1.35)
Guinea-pig...	...	...	...	...	1.16 (1.11)

2 % is applicable to the cataphoretic velocity of red blood corpuscles indicates that the surface potential of the corpuscle does not depend directly on the vitality of the cell inasmuch as the processes of the living cell are generally associated with a greater and less regular temperature coefficient. In this con-

nection an investigation of the temperature coefficient of cataphoretic velocity of the erythrocytes of some poikilotherms (*e.g.* the frog) would be of considerable importance, for comparison with the above homoeotherms could then be made.

An observation of some interest was made in a few experiments where the erythrocytes had partly haemolysed. In these experiments the intact cells migrated independently of the oxyhaemoglobin. The final result was the formation of three well-defined layers (which could be seen most clearly when a bright but diffused light was placed behind the apparatus) consisting of the colourless buffer, the transparent red solution of oxyhaemoglobin and the opaque suspension of intact cells. The oxyhaemoglobin showed no marked tendency to migrate: a slight wandering occurred sometimes to the cathode and sometimes to the anode. It should be noted that Geiger [1931] has claimed the isolation by cataphoresis of two different oxyhaemoglobins from the blood of some animals.

The opinion has been expressed that the surface potential of the erythrocyte appears to be a function solely of the constituents of the surface membrane and not to be correlated with the vital processes occurring at the interface. This is substantiated by the normal values obtained for the velocities of red corpuscles of the blood of mice suffering from arsenical poisoning. Arsenic-containing substances are well known to inhibit the oxygen uptake of certain tissues, and Onaka [1911] has recorded that arsenite, like cyanide, strongly inhibits the oxygen uptake of avian erythrocytes. Unfortunately facilities were not at hand to determine whether the oxygen uptake of the corpuscles actually used was below normal.

The cataphoretic velocity of mouse erythrocytes is but little changed by the presence of 0.1 % glucose or of 0.1 % glucose together with 0.02 % insulin. Abramson recorded an increase from 1.35 (in plain phosphate buffer at p_H 7.38) to 2.60 (in isotonic glucose and buffer at p_H 6.80). With mice suffering from hypoglycaemic convulsions there occurs a decrease in the velocity due to the presence of both glucose and insulin. This finding is of considerable importance and will again be referred to in the consideration of the rôle of the erythrocytes in the action of insulin.

From the above it is clear that the use of a macro-apparatus gives fairly constant and accurate results for the cataphoretic velocities of mammalian erythrocytes. This view leads to a fair degree of confidence in the undertaking of an investigation of the cataphoresis of human erythrocytes and their part in the action of insulin in the organism, an investigation which will be described in a further communication.

SUMMARY.

Values for the cataphoretic migration of mammalian erythrocytes (rat, rabbit, mouse, guinea-pig) at 22–27° in *M*/15 phosphate buffer were determined by means of a macro-apparatus and the results compared with those obtained by Abramson using a micro-method.

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