THE CATAPHORETIC VELOCITY OF MAMMALIAN RED BLOOD CELLS.

By HAROLD A. ABRAMSON.

(From the Laboratory of Research Medicine, Medical Clinic, the Johns Hopkins University, Baltimore.)

(Accepted for publication, March 20, 1929.)

INTRODUCTION.

The cataphoretic velocity of the red blood cells of different mammals is determined by certain surface characteristics of the cells and hence is of value in studying the constitution of the cell surface. The correlation of these values with the zoological classification of the animals may have phylogenetic or ontogenetic significance. The cataphoretic velocities of the red blood cells of a group of mammals is the subject of this communication.

HISTORICAL.

There have been a great many experiments dealing with the sign of charge of red cells and the order of speed with which the red cells of different mammals move in an electric field. These experiments, however, must be considered for the most part as qualitative, and when fine distinctions are to be drawn, very frequently erroneous. Kozawa (1) noted that the red cells of different animals could be brought to an isoelectric point at a pH characteristic for each animal, the pH decreasing in the order

Rabbit > Guinea pig > Cat, Human > Dog > Pig.

Less acid was required if the suspensions were allowed to stand. It was evident to Kozawa that some change with time took place in the suspensions which altered the surface of the red cells. Kosaka and Seki (2) have also attempted to compare the red cells of different animals. In 0.9 per cent NaCl (the pH is not given) they found the qualitative series, (order of increasing velocity)

Rabbit < Pig < Guinea pig < Man < Mouse < Rat, Cat, Dog.

If the suspending fluid was 9.5 per cent saccharose instead of saline the order just cited was reversed to

Cat, Dog < Man < Rat < Guinea pig < Pig < Rabbit.

Here again there is no mention of degree of acidity. Kosaka and Seki state further that the red cells of the rabbit in serum move to the cathode. Such data show that there probably are differences, but they cannot be used quantitatively. Kozawa's observations, namely, that the surfaces of red cells in acid media undergo an important change easily perceptible by the method of cataphoresis were confirmed and extended by Eggerth (3) and also by Netter (4). Eggerth also found that human cells at pH 5.2 and below, particularly in buffered sugar solutions, show a marked change in velocity on standing. Netter found the same for ox and horse red cells. This change occurred whenever cells remained for some time in a solution of low salt concentration. It is evident that experiments which deal with differences in red cells of different animals in acid solutions are not easily interpreted because the acid produces an unstable system at the red cell surface. One might, perhaps, speak of a "dying red cell" with death or "equilibrium" at the end of 2 hours immersion—the time at which Netter considered "equilibrium" to be present. One may properly compare red cells of different animals in a given medium only if there is no change with time. Netter found that horse red cells in serum and in buffered saline solutions moved slightly faster than ox cells in the same medium. Bernardi (5) has found the following series:

Rabbit < Cat < Guinea pig < Man < Pig.

Much of the data cited above have been obtained under uncertain experimental conditions and with practically complete disagreement amongst the various authors.

Freundlich and Abramson (6) have found that red and white cell velocity in serum did not vary very much in 10 different adult horses, although differences between red cell and white cell velocity were marked. Abramson (7) has published similar data for red and white cells in plasma of 7 different horses.

METHODS.

The method of measurement and of calculation of absolute cataphoretic velocity was performed in the manner described by Abramson (8). The apparatus employed was a modification of that first published by Northrop and Kunitz (9) and Kunitz (10). A single apparatus was used throughout. The values of mobility for this cell corresponded within 9 per cent of values determined by means of a cemented cell of the same general construction, but of different dimensions. Mobility values found by Abramson (11) for quartz particles covered by albumin in this cemented cell have been checked by the streaming potential measurements of Briggs (12) on similar surfaces of quartz membranes covered with the same protein. The velocities here given for red cells, having been checked directly and indirectly, should represent absolute mobilities certainly within 10 per cent. It will later appear that smaller differences may, on occasion, be extremely simply estimated.

The blood cell suspensions were made up as follows. One drop of freshly drawn blood (in the capillary portion of a white cell pipette) was added to 25 to 50 cc. of M/15 phosphate buffer of pH = 7.35 ± 0.03 so that an exceedingly dilute suspension of blood cells was obtained. As will appear later, the volume of the blood added, within reasonable limits, does not interfere with the constancy of cataphoretic velocity of the blood cells. The buffer employed had a specific resistance of 126 ± 1 Ohms at 25°C. Measurements of cataphoresis were made at room temperature, which usually varied between 22° and 27°C. The values in the tables are corrected to 25° by assuming that the temperature coefficient of the cataphoresis of the red cell in the medium employed is 0.02 per degree Centigrade.

The type of cataphoresis cell used is readily cleaned with cleaning mixture between measurements. This precaution was not taken, however, as experiment proved it to be unnecessary. Running tap water was drawn between measurements for 2 minutes through the cell by means of a suction pump. If it be desired to study the effect of short periods of time on mobility the cell can be turned upside down, and the particles resuspended.

EXPERIMENTAL.

The data discussed in the historical section were obtained for the most part under conditions which did not lead to a steady state without necessitating a destruction of the red cell structure. Before the cells of different animals could be compared, therefore, it was essential that a medium be employed which had no destructive effect on the cell. M/15 phosphate buffer of pH 7.4 was chosen. Table I gives the effect of time of standing on mobility. It is evident that for a large variety

TABLE I.

The Effect of Standing Time on Velocity.

There is no significant change under the experimental conditions.

Animals	Velocity at first	Velocity after standing
	μ per sec. per volt per cm.	*μ per sec. per volt per cm.*
Man 1	1.21	1.12 (12 hrs.)
" 2	1.22	1.22 (48 hrs.)
" 3	1.23	1.20 (18 hrs.)
" 4	1.30	1.29 (18 hrs.)
" 5	1.20	1.15 (24 hrs.)*
" 6	1.28	1.18 (24 hrs.)*
Opossum	1.05	1.11 (24 hrs.)
Dog	See Table III	1.55 (12 hrs.)
Cat	See Table III	1.39 (12 hrs.)
Sloth	0.97	1.00 (24 hrs.)
White rat	See Table III	1.43 (24 hrs.)

* Sickle cell anemia.

of animals (man, dog, cat, sloth, opossum, rat) the cataphoretic velocity remains unchanged in this medium for at least 24 hours. A second matter to be considered was the effect of further dilution and of repeated washing of the red cell suspensions. The suspensions studied, as Table II demonstrates, were as dilute as necessary to obtain constant values. Washing with large volumes of buffer produced no significant change in the velocities of rabbit and human cells. It is to be noted that almost the same constant value was obtained if washed cells were hemolyzed by diluted hypotonic buffer and cells subsequently added to the hemolytic mixture (which had been again made isotonic). This confirms a similar observation made by Eg-

gerth: that red cells adsorb little if any hemoglobin in media at this reaction.

The Cataphoretic Velocity of Normal Adult Human Cells.

A series of 10 normal white adults gave a mean velocity of 1.31 ± 0.02 μ per second per volt per centimeter. Both sexes were about equally represented and the ages varied from 19 to 53 years.[1] The mean of 10 normal adult Negroes velocity was 1.30 ± 0.05μ per second per volt

TABLE II.

10 cc. of a dilute suspension of red cells whose volume per cent was less than 2 per cent were washed at least 5 times with 10 cc. of N/15, pH 7.4 phosphate buffer. The residual volume was about 0.3 cc. The data are not corrected to 25°C. There is no easily perceptible change.

Exp.	Animals	Velocity before washing	Velocity after washing	Remarks
		μ per sec. per volt per cm.	μ per sec. per volt per cm.	
1	Man	1.14	1.20	
2	Man	1.27	1.29	
3	Man	1.31	1.31	Hypertension Nephritis Secondary anemia
4	Rabbit	0.50	0.52	
5	Human cells washed as above, then hemolyzed; similarly washed cells then added. See text	See table for limits of normal	1.20	
6			1.34	

per centimeter. Three members of the Yellow race possessed similar velocities in comparable experiments. This is indirectly confirmatory of the observations of Schroeder* who noted that there were no differences in speed amongst red cells of the various Landsteiner blood groups (Table III). While it is relatively simple to get values corresponding within 3 to 5 per cent on the same day, errors of meas-

* Schroeder, *Pflug. Arch. f. d. ges. Physiol.*, 1926, ccxv, 32.

[1] Another series of 10 white adults in a buffer having a slightly greater salt content gave a value of 1.22 ± 0.02 per second per volt per centimeter.

urement from day to day lead to wider variations as is evident from Table IV where the measurements were made during several weeks

TABLE III.

The Cataphoretic Velocity of Mammalian Red Cells at pH 7.35 in M/15 Phosphate Buffer.

The velocity of a human control is given only when small differences are to be considered.

Order	Animal	Number of animals investigated	Mn. observed velocities	Average deviation	Velocity of human control	Remarks
			μ per sec. per volt per cm.		*μ per sec. per volt per cm.*	
Primate	Man (white)	10	1.31	±0.02		
	Man (negro)	10	1.30	±0.05		
	Monkey (*Macacus rhesus*)	4	1.25	±0.02	1.23	2 young monkeys
Carnivor	Dog	3	1.68	±0.03		Fairly rapid hemolysis
	Cat	3	1.40	±0.01	1.32	Fairly rapid hemolysis
Ungulate	Pig	5	0.98	±0.03		Rapid spontaneous hemolysis
Rodent	Rabbit	5	0.55	±0.05		Included 2 young rabbits
	Guinea pig	6	1.11	±0.02		
	Mouse	5	1.35	±0.06	1.26	4 white mice
	Rat	5	1.45	±0.02	1.31	4 white rats
Edentate	Sloth	1	0.97			Two-toed sloth One series of measurements
Marsupial	Opossum	1	1.07	±0.02*		Adult, male

* Two series of measurements.

on a single individual. Although the mean is practically the same within the limits of error, it is evident that for fine differences in velocity between different animals a comparison must be made with a

control under as similar experimental conditions as possible. The ideal arrangement would be to study cells from different animals simultaneously in the same suspension. As will appear shortly, this is possible.

Normal White Infants.—Table V demonstrates that the cataphoretic velocity of the red cell in the new-born white infant is similar to that in the adult. During the extra-uterine life of the white race, therefore,

TABLE IV.

Measurements Repeated over 7 Weeks Showing Variation in Control.

Date	Velocity
	μ per sec. per volt per cm.
Oct. 18	1.29
23	1.26
24	1.22
26	1.30
28	1.21
30	1.19
Nov. 2	1.25
6	1.31
7	1.37
11	1.28
12	1.37
16	1.29
17	1.29
21	1.26
26	1.35

Mn = 1.28 ± 0.04.

the surface of the red cell remains, on the whole, the same. It is evident that the problem may be extended to the study of changes during intra-uterine development.

Mammals.—The data given in Table III clearly show that very wide differences in the cataphoretic velocity of the red cells of different mammals exist. The absence of an effect of age in humans has been noted. Cells of young monkeys and young rabbits had the same speed as the adults. The mean value of 0.55 per second per volt per centimeter found for the rabbit is but one-third of the speed of dog cells. In the series here presented the following order is found.

Rabbit < Sloth, Pig < Opossum, Guinea pig < Man, Monkey < Mouse, Cat, Rat < Dog
(0.55) (0.97) (0.98) (1.07) (1.11) (1.31) (1.33) (1.40) (1.39) (1.45) (1.65)

The number under each type of animal is the value in μ per second per volt per centimeter taken from the tables but corrected for the value of the controls when small differences are present. Thus for monkey the speed found was 1.25 per second. The control (human) was 1.23 per second. Taking 1.31 per second as the standard speed, the speed for the monkey becomes 1.33μ per second.

TABLE V.

Normal White Infants.

Baby	Age	Velocity
		μ per sec. per volt per cm.
Series I:		
No. 1	9 days	1.28
Control adult		1.22
Series II:		
No. 2	5 days	1.23
No. 3	3 hrs.	1.18
No. 4	2 days	1.23
No. 5	1 hr.	1.18
No. 6	1 day	1.27
No. 7	1 day	1.22
Control adult		1.21
Mn. infants (Series II)		1.25

It is to be emphasized that these values are for the particular experimental conditions described above. It is known that changes in pH or of salt concentration or valence can produce changes in the cells of a single type of animal greater than the variations described here for the cells of different animals.

This series has been confirmed by comparing red cells of different animals in the same medium at the same time. If rabbit cells are mixed with human cells, it is quite striking to see the human cells, which move more than twice as fast as those of the rabbit overtake

and pass the rabbit cells. In this manner it can be clearly demonstrated that mouse cells, for example, move faster than human cells. The smaller mouse cells may be easily distinguished from human cells and the greater speed of the former easily perceived. The presence of high concentration of electrolytes makes measurements less precise than in more dilute solutions because of the low particle speed and because of the increased electroendosmotic flow, with turbulence more readily occurring. The differences between mouse and human cells which have been measured in successive measurements are at the limits of the experimental error. It was consequently observed with satisfaction that these slight differences which had been measured could be confirmed by direct simultaneous observation. The fact that small amounts of heterologous plasma does not change the red cell surface to an appreciable extent under these conditions is in keeping with the previous observation that red cells can migrate through gelatin serum sols and gels without adsorbing appreciable amounts of protein (13). These experiments can be confirmed by other striking experiments which demonstrate that the red cell preserves its surface integrity. Human red cells were added to a suspension of rabbit cells in rabbit blood (diluted 1:4 with isotonic glucose). In this mixture, which contained about 2 per cent of rabbit plasma proteins, human cells moved about twice as fast as the rabbit cells. And similarly, in the converse experiment, rabbit cells in human serum (diluted 1:6 with isotonic glucose) migrated with about one-half the speed of human cells. This fact may be of use in determining the fate of intravenously injected heterologous red cells. A simple experiment (Table VI) demonstrates that while cholesterol and quartz adsorb gelatin from dilute solution in M/15 phosphate buffer at pH = 7.35, the red cell even after 24 hours has not changed its cataphoretic velocity. The red cell surface is, therefore, here also practically unaffected by the proteins present.

These experiments do not demonstrate that union of red cell with heterologous serum protein does *not* take place at all. It seems likely, in view of preliminary experiments dealing with this point, that some combination does take place in high concentrations of serum with certain components of normal inactivated serum. Whether or not the reaction is similar to that which is supposed to accompany sensi-

tization is under investigation and will be described in a future communication.

Kosaka and Seki have noted that suspending the cells in isotonic sugar solutions reverses the order of magnitude of mobility. Table VII gives data in disagreement with these findings. The velocity

TABLE VI.

The Effect of Gelatin on the Cataphoretic Velocity of Quartz, Cholesterol and Red Blood Cells in pH 7.35 M/15 Phosphate Buffer.

Particle	Cataphoretic velocity	
	Without gelatin	With gelatin
	μ per sec. per volt per cm.	*μ per sec. per volt per cm.*
Human red cell	−1.31	−1.27 (after 24 hrs. 1.23)
Quartz	−3.55	−0.39 approximate
Cholesterol	−2.25	−0.35 approximate

TABLE VII.

Red Cells Suspended in Isotonic Dextrose Solution and Dilute Phosphate Buffer (pH = 6.8).

Each value is the mean of two determinations on two animals.

Animal	Velocity	Remarks
	μ per sec. per volt per cm.	
Rabbit	1.27	
Pig	2.09	Marked hemolysis
Man	2.33	
Mouse	2.60	

order Rabbit < Pig < Man < Mouse is, as in pure phosphate buffer, also to be found in suspensions of the red cells of these animals in phosphate buffered isotonic sugar solutions.

There is no relationship between our series and the ease with which hemolysis occurs spontaneously. Pig cells are most fragile in M/15 phosphate buffer. Human and rabbit cells hardly hemolyzed at all. Cat and dog cells show a marked tendency to hemolyze.

There was no agglutination within 24 hours; nor did slow centrifugation produce agglutination.

TABLE VIII.

Patient	Age	Race	Sex	Clinical diagnosis	Velocity
					μ per sec. per volt per cm.
1 M.	19	W	F	9 mos. pregnancy	1.35
2 H.	19	W	F	9 mos. pregnancy	1.31
3 P.	34	W	F	9 mos. pregnancy	1.31
4 C.	19	W	F	9 mos. pregnancy	1.30
5 Mc.	26	W	F	Secondary anemia	1.34
6 J.	42	W	M	"Cured" (liver diet) Pernicious anemia	1.30
7 A.	32	B	F	Severe secondary anemia	1.12 (control 1.25)
8 M.	52	W	M	Nephritis; hypertension mild secondary anemia	1.34
9 P.	52	B	F	Carcinoma of colon; mild secondary anemia	1.17 (control 1.26)
10 S.	7	B	M	Sickle cell anemia	1.28
11 J.	8	B	F	Sickle cell anemia	1.20
12	3	B	M	Sickle cell anemia	1.28
13 H.	58	W	M	Pernicious anemia	1.20 (cells of different shape have a same speed)
14 F.	30	W	M	Severe secondary anemia (6 transfusions during 10 wks. previous to admission)	1.25
15 M.	20	W	F	Severe $HgCl_2$ poisoning. Very severe anemia (citrate transfusion previous day)	1.24
16 M.	45	W	F	Pernicious anemia carcinoma. Very severe anemia	1.35 (transfusion day before. Normal cells in blood suspension)

The approximate ζ-potential may be obtained by multiplying the values given in the table by the factor 13. The rabbit cells, the lowest of the series, have a ζ-potential of about 7 millivolts, while the dog cells

ζ-potential is about 21 millivolts. (Assuming dielectric constant = 80, viscosity = 0.01 for M/15 phosphate buffer at pH = 7.35).

The Red Cell in Pregnancy.

In four cases of pregnancy at term, the cataphoretic velocity of the red cells was normal. This suggests that red cells of pregnant women suspended in citrate (as in the sedimentation test) would have the same mobility as normal women, and that there is here no direct relationship between cataphoretic velocity and sedimentation velocity. This viewpoint is well borne out by related data of Netter. Netter found that horse red cells migrate very slightly faster than ox cells. Horse cells clump quickly and settle out almost at once. Ox cells on the other hand are noted for their stability in suspension without clumping. There is here evidently no relationship between low values of mobility and aggregation. The experiments of Northrop and Freund (14) and of Oliver and Barnard (15) should be consulted in this connection.

The Diseases of the Blood.—A short series of primary and secondary anemias were studied. In primary and secondary anemias, with the exception perhaps of Patient 7 A., no significant change in cataphoretic mobility has been observed. Three cases of sickle cell anemia had practically normal velocities. It is very striking to see all the cells, in anemias where extreme variations in size, shape and hemoglobin content of the cells exist, migrate with practically the same speed. Patient 16 M. had a transfusion the day previous to the examination. There were many normal, probably transfused, red cells to be seen moving with the same velocity as the distinctly abnormal cells. These observations also again demonstrate the obstinacy with which the red cell maintains the general integrity of its surface in spite of most varied circumstances.

DISCUSSION.

The recent experiments of Abramson (13), (16) in collaboration with Freundlich and with Michaelis have made it clear that neither the size nor the shape of microscopic particles suspended in electrolyte-containing media influences the cataphoretic velocity. The differences in red cell velocity given in the preceding statement are, there-

fore, almost certainly representative of the surface constituents of the different red cells. With this in mind the data presented may be discussed.

It is well known that the chemical make-up of the surface of the microscopic particle influences its cataphoretic velocity. And it is of importance to be able to ascribe the variations in velocity found amongst mammalian red cells to a correlated series of changes in the structure of the surface layers. It is more or less customary to consider the red cell surface as hydrophobic. The data of the Mudds is most pertinent and interesting (17). Yet Netter has proposed that the surface of red cells of the horse and the ox, for example, may be composed chiefly of protein. He concluded from cataphoretic experiments that the former was made up primarily of globulin while the latter was chiefly albumin. It is difficult to evaluate Netter's conclusions for his data were primarily obtained in acid solutions where, we have seen, secondary changes always occur. The large differences in mobility found amongst the various mammals, the speed of the rabbit cells of the order 0.55μ per second per volt per centimeter at the one extreme and the dog cells 1.65μ per second per volt per centimeter, at the other extreme, indicate that it is probably not strictly permissible to speak of a characteristic red cell surface in general. The speed of the rabbit cells is very close indeed to the order of magnitude of the speeds found for inert particles covered with protein. The dog cells, on the other hand, are very near the speed of 2.2μ per second per volt per centimeter given here for cholesterol. It is not intended to suggest that cholesterol is a constituent of the red cell surface but rather that substances of a somewhat similar constitution plus protein could account for the high mobility of the dog cell, with mixtures of protein plus this substance or substances producing the intermediate velocities. It may also be mentioned that there is scarcely any quantitative data on the absolute mobility of the plasma proteins and the common lipids.

Grouping the animals used according to order shows clearly that zoological Order and cataphoretic velocity do not invariably go hand in hand. Thus for the Rodents, the rabbit, guinea pig and mouse differ widely, the mouse cells having velocity values much closer to those of the Primates and Carnivora. The most striking observation

is the slow speed in the case of the rabbit cells compared with any other of the animals investigated.

The human red cell when examined in a suitable medium will probably be an excellent particle for the checking of the calibration of cataphoresis cells.

SUMMARY AND CONCLUSIONS.

1. No significant change with time (to 24 hours) in the cataphoretic velocity of certain mammalian red cells occurs when the cells are suspended in M/15 phosphate buffer at pH = 7.35. Neither successive washings nor standing effect a change.

2. In M/15 phosphate buffer at pH 7.35 ± 0.03 the following order of red cell velocity has been obtained. The numbers in parenthesis are μ per second per volt per centimeter.

Rabbit	<	Sloth,	Pig	<	Opossum,	Guinea pig	<	Man,	Monkey	<	Mouse,	Cat,
(0.55)		(0.97)	(0.98)		(1.07)	(1.11)		(1.31)	(1.33)		(1.40)	(1.39)
						Rat	<	Dog				
						(1.45)		(1.65)				

The order, though not the absolute values, was the same in buffered isotonic dextrose. Human and rabbit cells showed similar differences when both were studied simultaneously in the serum of either. Under these conditions, there is no apparent relationship between zoological Order and cataphoretic velocity.

3. Cholesterol and quartz adsorb gelatin from dilute solution in the phosphate buffer. Red cells, on the other hand, even after 24 hours contact with gelatin solution, retain their previous velocity.

4. Pregnant and non-pregnant white female humans have the same red cell cataphoretic velocity. (The cells were not agglutinated.)

5. In a series of severe anemias no significant change in cataphoretic velocity was in general apparent, although marked changes in the morphology of the red cells were present.

I am indebted to Professor L. Michaelis for much valuable advice received in connection with this investigation.

Through the kindness and cooperation of Professors Hartman, Richter, Longcope and of Dr. M. Smith much of the material investigated was made accessible.

BIBLIOGRAPHY.

1. Kozawa, *Biochem. Z.*, 1914, lx, 146.
2. Kosaka and Seki, Communications of Okayama Medical Society, 1921.
3. Eggerth, *J. Gen. Physiol.*, 1924, vi, 587.
4. Netter, *Arch. ges. Physiol.*, 1925, ccviii, 16.
5. Bernardi, *Arch. sc. biol.*, 1926, viii, 1.
6. Freundlich and Abramson, *Z. physik. Chem.*, 1927, cxxviii, 25.
7. Abramson, *J. Exp. Med.*, 1927, xlvi, 987.
8. Abramson, *J. Gen. Physiol.*, 1929, xii, 469.
9. Northrop and Kunitz, *J. Gen. Physiol.*, 1925, vii, 729.
10. Kunitz, Referred to by Mudd in Colloid Symposium Monograph No. 6, 1928, p. 131.
11. Abramson, *J. Am. Chem. Soc.*, 1928, l, 390.
12. Briggs, *J. Am. Chem. Soc.*, 1928, l, 2358.
13. Abramson, Colloid Symposium Monograph No. 6, New York 1928, p. 115.
14. Northrop and Freund, *J. Gen. Physiol.*, 1924, vi, 603.
15. Oliver and Barnard, *J. Gen. Physiol.*, 1925, vii, 99.
16. Abramson and Michaelis, *J. Gen. Physiol.*, 1929, xii, 587.
17. Mudd and Mudd, *Biochem. Z.*, 1927, clxxxvi, 378.