A COMPARISON OF THE ELECTROPHORETIC MOBILITIES AND SEDIMENTATION VELOCITIES OF RED CELLS FROM NORMAL AND PREGNANT HUMAN SUBJECTS[1]

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It has long been known that the extent to which red cells aggregate (as measured by sedimentation velocity) in their own plasma varies greatly with the species. Also, within one species (human) the degree of aggregation varies with the physiological or pathological state of the individual, being greatly increased above the normal during pregnancy and in many febrile and neoplastic disorders. It has recently been shown (1) that the main cause of species variations in sedimentation velocity is a difference in the red cells themselves, rather than in the plasma, since cells from various species sink at very different rates in a common medium such as 1 per cent gelatin. This species difference is correlated, 1, with the electrophoretic mobility of the red cells, the cells which settle most rapidly having the highest mobility at pH 7.4, 2, with the isoelectric point, the cells with highest sedimentation velocity having the lowest isoelectric point, and, 3, presumably with the chemical composition of the red cell surface, those cells sinking most rapidly which have the highest proportion of lipoid to protein with a resultant higher interfacial energy.

The increased rate of sedimentation accompanying pregnancy and pathological conditions in man, on the other hand, has long been assigned to an increase in the globulin and particularly in the fibrinogen fraction of the plasma. That an increase in plasma globulin takes place during these conditions has been repeatedly demonstrated; that such an increase in globulin concentration will directly affect the sedimentation velocity of the red cells is equally certain (2). The possibility of a difference in the cells of slowly and rapidly settling human blood comparable to that observed in different species has not, however, been excluded. Fåhraeus (2) observed the sedimentation of cells from normal males, in plasma from pregnant individuals, and *vice versa* and concluded that differences in the rate of aggregation and sinking are chiefly dependent on the properties of the plasma. The corpuscles from pregnant individuals, however, always sank somewhat more rapidly in a given plasma than the cells from males.

In order to avoid any group specific agglutination we have compared the sedimentation velocities of cells from normal and pregnant individuals in a common artificial medium, namely, 1 per cent gelatin, as well as in their own plasmas. Measurements of the electrophoretic mobility of cells from normal and pregnant people in a buffer medium at pH 7.4 were also made in order to detect, if possible, differences in the chemical make-up of the cells from such sources (1).

EXPERIMENTAL

Fresh oxalated blood, not more than 3 hours old, was used. All the blood samples for a single experiment were centrifuged at the same time and the sedimentation velocities measured in the same sample of 1 per cent gelatin. Determinations of electrophoresis were made in a cylindrical cell of a modified Mattson type. Observations were at a level of 0.147 cell diameter below the cell roof, at which level there is no electroosmotic movement. The visual axis was the vertical diameter of the cell. A field strength of 9.1 volts per centimeter was used; five observations of red cell movement for each direction of current were made, and the average velocity computed. The cells were suspended in M/50 phosphate buffer of pH 7.4 plus 0.3 per cent NaCl plus 6 per cent sucrose, the latter being substituted for part of the salt to cut down current density.

The 1 per cent gelatin used in the sedimenta-

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tion experiments was made up in M/50 phosphate buffer at pH 7.4 plus 0.9 per cent NaCl. The cells were centrifuged for fifteen minutes at 2000 r.p.m. and 2 volumes of the gelatin solution or autogenous plasma added to 1 of packed cells. The thoroughly mixed cell suspensions were sucked up into glass tubes of 3.5 mm. bore and 35 cm. long. The tubes bore short segments of rubber tubing on their lower ends which, after filling, were closed with spring clips. The number of millimeters which the cells sank in a specified time was measured. The precautions of maintaining a constant ratio of cells to volume of the suspension medium and of having the tube sufficiently long that the rate of sedimentation is not slowed by a beginning packing are essential to quantitative comparisons. The former precaution is practically always and the latter often disregarded in clinical work.

The results are shown in Table I. Only the measurements within a given experiment are comparable, since on different days the solutions used and conditions such as temperature might vary. It is evident that no measurable difference exists between the electrophoretic mobilities of cells from normal and pregnant individuals. This confirms the work of Abramson (3). Fåhraeus' (4) original statement that cells from male subjects show a greater mobility than those from pregnant subjects was admittedly based upon non-quantitative observations and was apparently retracted by him three years later (2), although this point has frequently escaped notice in the subsequent literature.

It is also evident that, whereas cells from pregnant people sink much more rapidly (from 3 to 25 times) than cells from non-pregnant individuals in their own respective plasmas, there is little or no difference between the sinking velocities of the two types of cells in 1 per cent gelatin. In Experiment 1 there is no correlation between the condition of the subject from which the cells came and the sedimentation velocity of the cells in gelatin. In Experiment 2 the slight (40 to 50 per cent) increase in sedimentation of the pregnant over the normal cells in gelatin is probably to be ascribed to the effect of the globulin-rich plasma remaining in the packed cell mass after centrifugation. This conclusion is supported by the findings of Experiment 3, in which the cells previously to being suspended in 1 per cent gelatin were washed with 0.9 per cent sodium chloride solution to free them of plasma. It is seen that the cells from the pregnant subjects sank at the same rate in 1 per cent gelatin as did those from the normal non-pregnant ones. Differences observed are not sufficiently great to warrant the conclusion that a chemical difference, comparable to that demonstrated for different species, exists in the cells of normal as compared with those of pregnant human subjects.

TABLE I

Sedimentation velocity and electrophoretic mobility of cells from pregnant and non-pregnant human subjects

Condition of subject	Electrophoretic mobility	Sinking in 1 per cent gelatin	Sinking in autogenous plasma
	micra per second per volt per cm.	*mm. in 30 minutes*	*mm. in 60 minutes*
Experiment 1			
Normal male	1.22	87	3
Normal female	1.25	74	8
Normal male	1.22	103	3
At term	1.23	120	76
At term	1.24	99	43
14 hours postpartum	1.25	55	17
		mm. in 20 minutes	*mm. in 30 minutes*
Experiment 2			
Normal female	1.39	81	4
Normal female	1.42	81	5
Normal female	1.40	70	6
At term	1.46	128	60
At term	1.39	117	30
At term	1.37	111	49
		mm. in 20 minutes	*mm. in 30 minutes*
Experiment 3			
Normal female	1.24	39	8
Normal female	1.27	49	1
Normal female	1.25	35	9
At term	1.27	40	55
At term	1.24	42	31
5 hours postpartum	1.28	31	28

SUMMARY

Whereas red cells from pregnant human subjects sink in their own plasma from 3 to 25 times more rapidly than do cells from normal people in their own plasma, no consistent difference is found in the respective sedimentation velocities

in a common medium, 1 per cent gelatin. Also, no measurable difference exists in the electrophoretic mobility of cells from normal as compared with that of cells from pregnant individuals in a common buffer medium. It is concluded that the increased sedimentation velocity of cells in blood from pregnant people is entirely due to changes that occur in the plasma during pregnancy.

BIBLIOGRAPHY

1. White, H. L., and Monaghan, B. R., Properties of red cell surfaces influencing rouleau formation. Am. J. Physiol., 1936, **115**, 31.
2. Fåhraeus, R., The suspension-stability of the blood. Acta med. Scandinav., 1921, **55**, 1.
3. Abramson, H. A., The cataphoretic velocity of mammalian red blood cells. J. Gen. Physiol., 1929, **12**, 711.
4. Fåhraeus, R., Über die Ursachen der verminderten Suspensionsstabilität der Blutkörperchen während der Schwangerschaft. Biochem. Ztschr., 1918, **89**, 355.