The Effect of the Plasma Proteins upon the Sedimentation Rate of Human Blood

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Fresh blood which contains an anticoagulant will separate out on standing, leaving a sharp boundary between the clear plasma above and the packed red cells below. The rate of this sedimentation is used as a clinical test, because in the healthy subject it occurs very slowly, while in many pathological conditions it is extremely rapid. From the work of Fahraeus [1929], Tiffeneau & Gysin [1937] and Frazer & Rennie [1941] it is known that the rate of sedimentation is determined by the protein constituents of the plasma, but no clear correlation has yet been found between the observed sedimentation rate and the quantities of the various protein fractions [Alred-Brown & Monroe, 1935; Ropes, Rossmel & Bauer, 1939; Frazer & Rennie, 1941]. In this paper the problem has been tackled by building up 'pathological plasmas' with protein fractions isolated from normal plasma and observing the behaviour of normal cells suspended in them.

METHODS

Estimation of sedimentation rate. The following procedure gave consistent results. Blood was collected into a tube containing dry anticoagulant in such amount that 100 ml. of blood contained 0.3–1.0 g. of Na citrate, or 0.5–3.0 g. of heparin. Within 15 min. of withdrawing the blood it was suspended in a Westergren type of tube, giving a column 200 mm. high and 2.8–3.0 mm. in diameter. This was fixed vertically in a rack, and the sedimentation rate expressed in terms of the height in mm. of plasma visible above the erythrocytes at a given time.

Preparation of protein fractions. The material used was normal human plasma which had been citrated and kept for about 6 days at 4°. The salted-out precipitates were dialysed under pressure against 0.85% NaCl until no contaminant salt remained, and the proteins sterilized by Seitz filtration and stored at 4°. Fibrinogen, total globulin, euglobulin, pseudoglobulin and total albumin were precipitated by appropriate concentrations of $(\text{NH}_4)_2\text{SO}_4$; crystallin, globoglycoid and seromucoid were separated as described by Hewitt [1938a], and by Rimington & van den Ende [1940], and nucleo-protein by the CO_2 precipitation method of Plimmer [1926]. All these fractions gave Molisch and cystine-S reactions in accordance with the literature. Seroglycoid could not be obtained, and if it does exist in human plasma it will be included in our total albumin fraction, and will not be present as a contaminant in any subsidiary fraction. Hewitt's seroglycoid may be equivalent to our seromucoid.

Our experiments were always duplicated, and usually repeated three or more times on different occasions, using

separate batches of the protein in question, and different normal cells. The variation in the results was usually less than $\pm 9\%$.

RESULTS

Influence of 'purified' plasma proteins on rate of cell sedimentation

(a) *Influence of mode of preparation of total globulin fraction on its action on sedimentation.* Fahraeus [1918], and later Tiffeneau & Gysin [1937], showed that if cells were suspended in solutions of protein fractions isolated from plasma, the sedimentation rates obtained were in the order, fibrinogen > globulin > albumin. Before attempting to repeat these results, experiments were carried out to ascertain whether the treatment undergone by a protein fraction during isolation affected its sedimentation-influencing properties. The results obtained with the total globulin fractions separated from portions of the same batch of normal plasma by three different methods (Table 1) showed substantial differences, despite the fact that the pseudoglobulin/euglobulin ratios were the same (4/1) for all three preparations.

Table 1. *Rate of sedimentation of human erythrocytes in the presence of serum globulin prepared by different methods*

(Total globulin conc. = 3 g./100 ml. 0.85% NaCl.
Cell vol. = 20%.)

Sedimentation after 30 min. (mm.)	Total globulin fractions prepared by precipitation by		
	Sat. with NaCl	Sat. with MgSO_4	$\frac{1}{2}$ -sat. with $(\text{NH}_4)_2\text{SO}_4$
	10	15	5

(b) *Action of single plasma proteins prepared as described under Methods.* We repeated the experiments of Fahraeus [1918] and of Tiffeneau & Gysin [1937], using a greater range of plasma protein fractions. From the results shown in Table 2, it will be seen that the most rapid sedimentation rates are obtained with fibrinogen and euglobulin. The question arose whether the 'slower' proteins, such as total albumin, when mixed with a 'fast' protein, merely reduced the sedimentation rate by acting as a diluent, or if the resulting sedimentation rate was the mean of those for the two separate proteins.

Table 2. *Rate of sedimentation of human erythrocytes suspended in 'pure' solutions of normal plasma protein fractions*

(Protein conc. = 3 g./100 ml. 0.85% NaCl.
Cell vol. = 20%.)

Protein	Average sedimentation (mm.) after			No. of exp.
	1 hr.	4 hr.	24 hr.	
Fibrinogen	100	150	150	3
Total globulin:				
(a) Pseudoglobulin/euglobulin ratio = 4:1	4	22	100	2
(b) Pseudoglobulin/euglobulin ratio = 1.6:1	14	20	130	2
Euglobulin	20	50	70	3
Pseudoglobulin	2	16	50	4
Total albumin	1.5	8	26	3
Crystallalbumin	2	10	40	2
Globoglycoid	2	5	26	1
Seromucoid	3	7	37	1
Nucleoprotein	3	8	40	2

(c) *Interaction of two plasma proteins.* For experiments with fibrinogen three tubes were set up containing (a) fibrinogen (3 g./100 ml.); (b) the same fibrinogen solution diluted with an equal volume of saline, and (c) the fibrinogen solution diluted with an equal volume of a 3 g./100 ml. solution of total albumin. The results (Table 3) show that albumin

Table 3. *Rate of sedimentation of human erythrocytes suspended in solutions of fibrinogen diluted with saline or albumin*

(Fibrinogen and albumin sol. = 3 g./100 ml. 0.85% NaCl.
Cell vol. = 20%. Results mean of 5 experiments.)

	Equal vol. of		
	Fibrinogen and fibrinogen	Fibrinogen and 0.85% NaCl	Fibrinogen and albumin
Sedimentation after 1 hr. (mm.)	100	40	6.0

does not act simply as a diluent; if the sedimentation rate in the mixture were the mean between that in fibrinogen (100 mm.) and that in albumin (1.5 mm.), the reading in the third tube should have been 50.7 mm. Actually the albumin inhibits the action of the fibrinogen so strongly that the reading is scarcely more than that given by a 3% solution of albumin alone. The action of other proteins upon fibrinogen was therefore studied, in order to ascertain whether this inhibitory action was peculiar to albumin.

The series of experiments in Table 4 shows that globulin slightly accelerates the sedimentation rate in fibrinogen solutions, and that this effect is due to the euglobulin fraction. The inhibiting effect of the albumin is caused by globoglycoid, while crystallalbumin and seromucoid have hardly more effect than a mere saline dilution. The effect of nucleoprotein

Table 4. *Sedimentation of human erythrocytes suspended in solutions of fibrinogen mixed with equal volumes of another protein fraction*

(Protein conc. = 3 g./100 ml. 0.85% NaCl.)

Protein mixture	Mean sedimentation at end of 1st hr. mm.	No. of exp.
Fibrinogen and saline	40	2
Fibrinogen and total globulin (pseudoglobulin/euglobulin ratio = 4:1)	60	2
Fibrinogen and euglobulin	75	2
Fibrinogen and pseudoglobulin	30	3
Fibrinogen and total albumin	4.6	4
Fibrinogen and crystallalbumin	50	3
Fibrinogen and globoglycoid	10	2
Fibrinogen and seromucoid*	70	1
Fibrinogen and nucleoprotein	1.0	3

* Seromucoid 1.8%.

is surprising, for after 24 hr. the fall is only 3 mm. Its normal concentration is low (0.003 g./100 ml. plasma), and since it is insoluble at 20% sat. $(\text{NH}_4)_2\text{SO}_4$, it will occur as a contaminant in the fibrinogen fraction.

These results suggest that the interaction of proteins in the plasma has a great influence upon erythrocyte stability. The effect of combining any two protein fractions in equal proportions has been worked out in some detail, and the corresponding saline dilutions given for comparison. The results tabulated in Table 5 are expressed as the fall at the end of 3 hr. with a 20% volume of fresh normal cells. The 3rd hr. reading is presented here because the 'slower' proteins fail to show significant differences at 1 hr., and the 'faster' have all reached the terminal packing stage by the 5th hr. To find the sedimentation for, say, a combination of fibrinogen and euglobulin, take the horizontal fibrinogen row of figures and follow it along until it intersects the vertical column for euglobulin. The reading in this square is 142 mm., which represents the average 3rd hr. fall for this combination. Table 5 shows the action of the proteins upon fibrinogen and also the inhibitory effect of total albumin upon the globulins, which is not so great in proportion as with fibrinogen. Nucleoprotein gives a greater sedimentation rate alone than when mixed with fibrinogen. We can give no explanation of this, but the experiment was repeated three times with the same result. The interaction between the 'slower' proteins is uninteresting. The figures show the effect of two proteins only in solution: in plasma, however, all the fractions are coexistent, and as we have shown that albumin inhibits fibrinogen, it seems likely that albumin will also inhibit the euglobulin which, if alone, would accelerate the fibrinogen, and thus exercise a double inhibition upon fibrinogen.

Table 5. *Sedimentation of human erythrocytes suspended in mixtures containing equal volumes of two plasma proteins*

(Protein conc. = 3 g./100 ml. 0.85% NaCl. Results given as mean of 2-4 experiments.)

	Mean sedimentation after 3 hr. mm.									
	Nucleo- protein	Sero- mucoid	Globo- glycoid	Cryst- albumin	Total albumin	Pseudo- globulin	Euglo- bulin	Total globulin	Fib- rinogen	Saline
Saline	—	—	3.5	—	5	7	10	7	97	3
Fibrinogen	3	130	40	100	10	60	142	130	150	97
Total globulin	—	—	6	7	7	9	20	11	130	7
Euglobulin	—	—	5	7	2	10	25	20	142	10
Pseudoglobulin	—	—	3	35	5	15	10	9	60	10
Total albumin	—	—	3	3	5	5	2	7	10	5
Crystalbumin	—	—	2	6	3	35	7	7	100	—
Globoglycoid	—	—	3	2	3	3	5	6	40	3.5
Seromucoid	—	5	—	—	—	—	—	—	130	—
Nucleoprotein	5	—	—	—	—	—	—	—	3	—

(d) *Interaction of three or more plasma proteins.*

This type of interaction was worked out in three stages:

(1) Fibrinogen altered: Constant globulin and albumin.

(2) Globulin and its fractions altered: Constant fibrinogen and albumin.

(3) Albumin and its fractions altered: Constant fibrinogen and globulin.

The effect of fibrinogen upon sedimentation rate was studied by comparing the fall of cells in plasma with that in serum from the same person, part of the specimen being collected with oxalate and part being allowed to clot for serum. The oxalated cells were centrifuged, washed in saline, and resuspended in the plasma and the serum. Table 6 shows that

Table 6. *Comparison of sedimentation rates of human erythrocytes suspended in plasma and in serum from the same blood*

(Five experiments performed.)

	Erythrocytes suspended in	
	Plasma	Serum
Normal blood, cell vol. reduced to 20%.	15	6
Sedimentation at 7th hr. (mm.)		
Pathological blood, cell vol. unaltered.	60	6
Sedimentation after 1 hr. (mm.)		

although some sedimentation can take place in the absence of fibrinogen, it plays the chief part, and therefore its acceleration or inhibition will have a great effect upon sedimentation. Oakley [1938] was able to add normal blood of known fibrinogen content to blood which completely lacked fibrinogen, and found that the relation between increased sedimentation rate and fibrinogen content was practically linear.

The second and third types of reaction were studied by constructing artificial plasma from a mixture of

single protein fractions. In order to test the accuracy of this method, sedimentation rates on normal and pathological blood were carried out parallel with analyses on the plasma, and records of cell size and cell volume kept. From these data artificial plasma was built up, the correct volume of fresh normal cells suspended in it, and the sedimentation rate determined. The total protein percentages were often lower than in the original, because it was found difficult to obtain all the individual fractions sufficiently concentrated, but the ratio of the component fractions was maintained. The protein solutions must be in good condition, for a denatured batch of, say, fibrinogen will invalidate the whole experiment. The results of imitating normal and pathological blood are shown in Tables 7 and 8.

Table 7. *Sedimentation of human erythrocytes suspended in natural or artificial normal plasma*

	Composition of natural normal plasma proteins as g./100 ml.	Composition of artificial normal plasma g./100 ml.
Fibrinogen	0.3	0.24
Globulin	2.7	2.16
Albumin	4.0	3.2
Total protein	7.0	5.6
Cell vol. (%)	40.0	40.0
Cell diam. (μ)	7.1	7.1
Sedimentation after 1 hr. (mm.)	4.0	3.0

From these two experiments we conclude that an artificial mixture of normal proteins does behave similarly to natural pathological plasma, in its effect upon sedimentation.

The globulin fractions were studied on the basis of the tuberculous plasma analysis given in Table 8. It will be seen that the fibrinogen and albumin were maintained constant and total globulin first replaced by euglobulin, and then by pseudoglobulin.

Table 8. *Sedimentation of human erythrocytes suspended in natural and artificial tuberculous plasma*

	Composition of plasma (proteins as g./100 ml.)			
	Artificial plasma containing			Natural plasma
	Total globulin	Eu- globulin	Pseudo- globulin	
Fibrinogen	0.6	0.6	0.6	0.6
Total globulin	3.32	3.2*	—	—
Euglobulin	2.15	3.2	0	—
Pseudoglobulin	1.17	0	3.2	—
Albumin	3.6	3.6	3.6	—
Total protein	7.52	7.4	7.4	—
Cell vol. (%)	40.0	40.0	40.0	40.0
Sedimentation after 1 hr. (mm.)	94.0	90.0	80.0	26.0

* Prepared by precipitation by MgSO_4 .

Because euglobulin slightly accelerates the sedimentation rate of fibrinogen (Table 4) we consider that the artificial plasma with euglobulin ought to have shown a greater sedimentation rate than total globulin, and attribute the slightly lower reading to a poor batch of the protein. The inhibitory action of the pseudoglobulin is, however, clear enough. Some months after this experiment was performed, a case of tuberculosis became available in which the sedimentation rate was low and the pseudoglobulin high, while the disease was sufficiently advanced for the sputum to show great numbers of tubercle bacilli. The results were as follows:

Plasma protein content		Conc. g./100 ml.	
Protein			
Fibrinogen	0.5	Cell vol. = 36 % Sedimentation (1 hr.) = 0.5 mm.	
Euglobulin	0.1		
Pseudoglobulin	2.3		
Albumin	4.49		

This analysis may well be compared with that for plasma and the artificial plasma containing pseudoglobulin (Table 8).

A typical nephritic blood was constructed in order to study the effect of the component fractions of albumin. This was chosen because there is much known about the albumin changes in nephritis and nephrosis. The fibrinogen and globulin were kept constant throughout; normal total albumin was used first to see if a typically rapid nephritic sedimentation could be obtained.

The sedimentation (Table 9) is not so great as one would expect in natural blood with the same composition. The total albumin was then replaced by globoglycoid and then by crystalalbumin. The plasma containing globoglycoid (Table 9) gave an extraordinarily slow sedimentation, and the

Table 9. *Sedimentation of human erythrocytes suspended in artificial nephritic plasma*

	Composition of artificial plasma (proteins as g./100 ml.) containing		
	Total albumin	Globo- glycoid	Cryst- albumin
Fibrinogen	0.32	0.26	0.32
Globulin	3.2	2.6	3.3
Total albumin	1.7	0.0	0.0
Crystalalbumin	0.0	0.0	1.6
Globoglycoid	0.0	1.3	0
Total protein	5.2	4.2	4.2
Cell vol. (%)	20.0	20.0	20.0
Sedimentation after 1 hr. (mm.)	15.0	1.0	145.0

crystalalbumin mixture caused a far more rapid fall than could be obtained in real nephritic blood. The interesting point is that the component fractions of albumin have a remarkably different effect upon the sedimentation rate. Unfortunately, seromuroid could not be prepared in sufficiently concentrated solution for use in these experiments.

Hewitt [1938*a*] analysed nephritic serum and found the crystalalbumin fraction to be far below normal, while the globulin and seroglycoid (equivalent to seromuroid?) were not strikingly different; no mention, however, is made of globoglycoid. If crystalalbumin is reduced in real nephritic blood, one would expect, from these experiments, a slower sedimentation rate. Unfortunately, there is no convenient method of analysing the albumin components on a small sample of blood, and our results do not tally with his findings.

DISCUSSION

From this series of experiments we conclude that the power of sedimentation of blood is not controlled by the absolute concentration of either the total plasma proteins or the protein fractions, but by *the inhibition of one protein by another*. Thus it is quite possible to have a high fibrinogen content and low sedimentation rate due to increase of pseudoglobulin at the expense of the euglobulin, while the total globulin concentration remains stationary; or an increase of globoglycoid over crystalalbumin can inhibit the action of the globulins without inducing any abnormality in the gross protein fractions. This concept probably explains why Alred-Brown & Monroe [1935] and Ropes *et al.* [1939] found no relation between the concentration of any one protein fraction and sedimentation rate, while Frazer & Rennie [1941] found decrease of albumin and, to a lesser extent, increase of fibrinogen, associated with rise of sedimentation rate, while the globulins had no appreciable effect.

Our experiments suggest that when the blood sedimentation rate is raised, there is no alteration in the chemical structure of the proteins. A possible exception to this is the Bence-Jones protein [Devine, 1941]. Geottsch [1936] found that nephritic total globulin was immunologically different from the normal, and Alving [1936] found changes in the cystine content of total albumin in nephrosis. Hewitt [1938*b*] later showed that in both cases the individual fractions of globulin or albumin give normal chemical and immunological reactions, and the altered ratios are responsible for the abnormality of the plasma.

SUMMARY

1. The proteins from normal plasma were separated into nine fractions, and the rate of fall of a

20% vol. of erythrocytes in 3% solutions of the proteins during 1 hr. was found to range from 100 mm. for fibrinogen to 1.5 mm. for total albumin.

2. The fractions were combined in equal amounts using 39 pairs. The action of the 'faster' fractions, fibrinogen and euglobulin, is inhibited by the 'slow' nucleoprotein and globoglycoid.

3. Artificial pathological plasmas built up from these fractions gave sedimentation rates similar to those for the comparable natural material. With artificial tuberculous plasma it was found that by altering only the component globulin fractions, the sedimentation rate could be changed from 94 to 26 mm.; and with artificial nephritic blood, substitution of total albumin by crystalalbumin altered the rate from 15.0 to 145 mm., while substitution of the total albumin by globoglycoid changed it from 15.0 to 1.0 mm.

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Phosphorolysis and Synthesis of Glycogen in Animal Tissues

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The mechanism of glycogenolysis in muscle and liver is described by Cori, Cori & Schmidt [1939] as follows:

- (1) Glycogen + phosphate $\rightleftharpoons$ glucose-1-phosphate (catalysed by phosphorylase and adenylic acid).
- (2) Glucose-1-phosphate $\rightleftharpoons$ hexose-6-phosphate (catalysed by phosphoglucomutase and Mg^{++}).
- (3) Hexose-6-phosphate $\rightleftharpoons$ glucose + phosphate (catalysed by phosphatase in liver).
- (3*a*) Hexose-6-phosphate $\rightleftharpoons$ hexose-1:6-diphosphate $\rightarrow$ lactic acid (in muscle).

These reactions are responsible for glucose formation in liver, and are the first steps in glycolysis in muscle. Glycogen resynthesis too is probably explained by the same reactions [Cori & Cori, 1941]. It seemed of interest, therefore, to study the distribution of the enzymes concerned, in various tissues at various stages of development, and to compare the enzymic activities with the rate of metabolism of glycogen in the tissue concerned. The further question was also investigated in this connexion whether insufficiency of one or more of the designated enzymes is the cause of metabolic disturbances in certain pathological conditions.

A separate series of experiments dealt with some properties of phosphorylase, and in particular with its inhibition by glucose. This inhibition was first observed by Lehmann [1938] who suggested its possible physiological importance. It may be responsible for the homeostatic mechanism described by Soskin, Essex, Herrick & Mann [1938].

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