ON THE CAUSES OF THE VARIATIONS IN THE
SEDIMENTATION OF THE CORPUSCLES AND
THE FORMATION OF THE CRUSTA
PHLOGISTICA ("SIZE," "BUFFY
COAT") ON THE BLOOD

A PRELIMINARY COMMUNICATION *

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If venous blood from a normal man or woman is left to clot it will, as a rule, form a homogenous red mass at the end of the coagulation.

In certain pathologic and physiologic states, the coagulum is differentiated into a lower red and an upper, more or less broad, yellowish layer (crusta phlogistica, buffy coat, size), which consists of coagulated plasma.

While this phenomenon is normal in the blood of several animals, it has been known from the earliest times that a crusta phlogistica in human blood is a pathologic phenomenon.

The newer medicine, however, generally has disregarded this symptom, possibly because it may be found in so many different states, which did not seem to form a unity. This opinion, for instance, is thus expressed by Panum¹ (1851): "Utrum crusta in sanguine appareat, necne, ex rationibus compluribus vere physicis pendet, et vix unquam licet inde certam sanguinis compositionem suspicari."

Investigations by Hewson² (1771) and Hunter³ (1794) had proved that a buffy coat on the blood appeared when the sedimentation of the corpuscles or the time of coagulation was increased. Both authors put stress on the changes of the clotting time.

A crusta phlogistica was found especially in cases of inflammation, so that Borsieri⁴ and Tommasini,⁵ who found this phenomenon in chlorosis, thought that this disease might be characterized as an inflammation.

Panum,¹ however, warned against repeated venesection in patients because their blood showed a crusta phlogistica, since this might be caused by the bloodletting itself.

Andral⁶ (1845) studied the conditions under which the crusta (couenne) formation took place clinically, and came to the conclusion

* From the Clinic of Professor Faber, Rigshospital.

1. Panum: Dissertation (Copenhagen). 1851.

2. Hewson: Experimental Inquiries in the Properties of Blood, London, 1771.

3. Hunter: Treatise on the Blood, London, 1794.

4. Borsieri: Loco incerto, quoted by Andral.⁶

5. Tommasini: Sull' infiammazione, quoted by Andral.⁶

6. Andral: Essai d'hématologie pathologique, Paris, 1845.

that a crusta on the blood appeared only when the fibrin percentage of the blood was increased or the number of corpuscles was decreased.

Henle,⁷ Dogiel,⁸ Panum,¹ Hayem,⁹ Valentin¹⁰ and Pfeiffer¹¹ all found that the sedimentation of the corpuscles was of extreme importance for the formation of the buffy coat, and that an increased sedimentation of the corpuscles generally was caused by an agglutination of the corpuscles.

Pfeiffer thought that an increased fibrinogen percentage might make the corpuscles sticky and thus further the agglutination.

Hekma¹² showed that the corpuscles of the horse sank more rapidly in citrated blood than in defibrinated blood, and de Haan¹³ found that the velocity of sedimentation was proportional with the degree of agglutination. The cause of the agglutination according to de Haan lies in the corpuscles, though he admitted that the medium of suspension had some influence.

Kozawa¹⁴ and Høber¹⁵ showed that there was a parallelism between the negative electrical charge of the corpuscles in different animals and the differences between the rapidity of sedimentation found by Berkzeller and Stanker.¹⁶

Schwyzler¹⁷ has investigated these relations more fully and finds that the suspension stability of the corpuscles depends on the negative charge of the corpuscles.

When this decreases, the corpuscles are attracted to one another and agglutinate. While citrate did not hamper the agglutination, a surplus of sodium chlorid (11 *pro mille*) prevented it.

Fåhræus¹⁸ specially studied the augmented sedimentation found during normal pregnancy. He found a proportionality between the sedimentation velocity and the degree of auto-agglutination.

The phenomenon, however, was not specific, since it was found also in most infectious diseases and in cases of cancer. Also the sedimentation was quicker in the blood of normal women than in that of normal men.

In a later paper Fåhræus¹⁹ discusses the causes of the changes in the stability of the blood, accepting the theory of the lowered electrical

7. Henle: *Loco incerto*.

8. Dogiel: *Arch. f. Anat. u. Physiol.*, 1883, quoted by Pfeiffer.

9. Hayem: *Du sang*, etc., Paris.

10. Valentin: *Pathologie des Blutes*, Leipzig, 1866.

11. Pfeiffer: *Ztschr. f. klin. Med.* **33**:215, 1897.

12. Hekma: *Biochem. Ztschr.*, Festband, p. 177, 1908.

13. de Haan: *Biochem. Ztschr.* **86**:298, 1918.

14. Kozawa: *Biochem. Ztschr.* **60**:146, 1914.

15. Høber: *Arch. f. d. ges. Physiol.* **101**:627, 1904.

16. Berkzeller and Stanker: *Internat. Ztschr. f. physiol. chem. Biol.* **3**:133, 1917.

17. Schwyzler: *Biochem. Ztschr.* **60**:297, 1914.

18. Fåhræus: *Hygiea*, 1918.

19. Fåhræus: *Biochem. Ztschr.* **89**:115, 1918.

charge as the reason for the augmented agglutination. He finds that the properties of the corpuscles are altered, but that the primary reason for this change is to be sought in the plasma.

As to the provocative cause, Fåhræus remarks that the sedimentation is increased in all cases in which Abderhalden supposes that proteids foreign to the blood are introduced into the circulation.

Quite recently Westergren²⁰ published a paper dealing with the rapid sedimentation found in cases of pulmonary tuberculosis. In this disease he constantly finds an increased sedimentation, beyond the normal values, when citrated blood is kept in narrow tubes and observed after one hour.

In this paper there is mentioned an as yet unpublished research by Fåhræus on the causes of the sedimentation.

While working on the causes of hemorrhagic diathesis, I have elaborated a technic,²¹ by which the fibrin percentage in blood and plasma may be determined by a gravimetric method on 2 c.c. cell-free citrated plasma obtained from 5 c.c. citrated blood.

The blood (4.5 c.c.) was taken into a graduated centrifuge tube (Oluf Thomsen) containing 0.5 c.c. of a 3 per cent. citrate solution. In many cases the platelet count (Thomsen,²² Gram²³) was determined by Thomsen's method and also the coagulation time of citrated plasma on recalcination (Gram²⁴). The tubes used were divided into 0.1 c.c., and the size chosen was such that 0.1 c.c. on the gradations equals 0.1 cm.

In order to calculate the platelet count and fibrin percentages, the cell volume was determined in all cases.

I soon found that there occurred a more or less pronounced sedimentation in these blood specimens, and that the sedimentation depended on (a) the cell volume percentage, and (b) the fibrin (fibrinogen) percentage in the plasma.

The sedimentation ordinarily was measured by observing the height in centimeters of the plasma layer formed, when the 5 c.c. citrated blood was left to stand at room temperature (about 19 C.) for ten minutes. The plasma layer was measured between the lower meniscus of the plasma surface and the red cell layer. Excepting in anemias, this borderline was nearly always quite clear and sharp as if cut with a knife.

20. Westergren: Acta med. scand. **54**:247, 1921.

21. The technic for the fibrin determinations will be published later, together with the results in each separate case.

22. Thomsen: Acta med. scand. **53**:507, 1920.

23. Gram: Arch. Int. Med. **25**:325 (March) 1920; Acta med. scand. **54**:1, 1920.

24. Gram: Bull. Johns Hopkins Hosp. **31**:364, 1920 (see corrections in the following issues).

In some ways it is most convenient to use an observation time of only ten minutes, but one must remember that cases with very different, generally normal fibrin percentages and cell volumes are thus included in the same group showing less than 0.1 cm. (i. e. traces) of sedimentation. However, in many pathologic cases the sedimentation after one hour has progressed as far as it will go, thus making the differences in these cases smaller than by an observation after ten minutes.

The formation of the crusta phlogistica was observed by letting 1 cm.³ of blood flow down in a miniature test tube having a diameter of from 9 to 10 mm., leaving it to clot in an upright position of the glass. The reaction could not be measured exactly, since the border between crusta and corpuscles generally is not very sharp. I have distinguished between: — (no crusta), traces, + (positive) and ++ (very broad).

The result of the cell volume determinations has been, that the cell volume in normal women oscillates about an average of 41 per cent. of the pure blood and between the limits 37 and 45 per cent.

In normal men, the cell volume oscillates about 48 per cent., and between the extremes 43 and 51 per cent.

A low cell volume, of course, was found in simple anemias, pernicious anemia and most cases of leukemia, pseudoleukemia and several other diseases of the blood. Some infectious diseases, as, for instance, rheumatic fever, typhoid, erysipelas and chronic tuberculosis, very often showed a low cell volume.

In cases of nephritis, and in cases of polyarthritis chronica and malignant tumors one may find a low cell volume. The same is true in normal pregnancy, which, especially in the earlier months, shows a distinct drop in the cell volume percentages.

The fibrin percentage per 100 cm.³ plasma—which is the value to be considered—in twenty-five normal individuals of each sex varied between 0.20 and 0.38 per cent., the mean values being 0.27 per cent. in men and 0.29 per cent. in women, that is, slightly lower in men than in women. These different levels of fibrin content are held from one time to another by the same normal individual.

In many chronic noninfectious diseases no deviations were found from the normal fibrin content of the plasma and the normal cell volume.

In the description of each separate disease, the number of cases observed and the average and variations of the fibrin percentage in plasma is added in brackets.

A fibrin percentage in plasma below the lowest normal limit was met with in some but not nearly in all cases of severe degeneration of the liver.

Out of fourteen such cases, six showed a fibrin percentage in plasma smaller than 0.20 per cent., and the average was 0.22 per cent. (variations from 0.39 to 0.07 per cent.). In some of these cases, when infectious complications were present, it appears that the normal fibrin percentage found must be considered pathologic. The diagnosis in all cases was verified postmortem.

Outside this group a fibrin deficiency was found only in a few cases of pernicious anemia²⁵ (fatty degeneration of the liver?) and one case of profuse carcinomatosis of the liver.

In simple anemia (fifteen cases, average 0.33 per cent., variations from 0.40 to 0.25 per cent.) the fibrin percentage in plasma was normal, though the percentage per 100 c.c. blood was very high.

In polycythemia (eight cases, average 0.30 per cent., variations from 0.40 to 0.21 per cent.) the percentage in plasma also varied nearly within the normal limits, while the percentage per 100 c.c. blood was very low. This means that the fibrin percentage in plasma, not that in blood, is the value which remains fairly constant, when the cell volume is altered.

In leukemia (fourteen cases, average 0.37 per cent., variations from 0.46 to 0.29 per cent.), especially in the myeloid type, one may find a slight absolute increase of the fibrin percentage in the plasma. The same is true in hemophilia (three cases, average 0.40 per cent., variations from 0.43 to 0.37 per cent.).

One case of purpura and one of pseudoleukemia showed normal fibrin percentages (respectively 0.31 and 0.34 per cent.), while a case of scurvy with gingivitis and loosened teeth had a slight increase (0.41 per cent.).

The increase is very pronounced and always absolute (i. e., > 0.38 per cent.) in lobar pneumonia (seven cases, average 1.05 per cent., variations from 1.50 to 0.80 per cent.), bronchopneumonia (five cases, average 0.75 per cent., variations from 1.29 to 0.52 per cent.), rheumatic fever (nine cases, average 0.77 per cent., variations from 0.94 to 0.55 per cent.), pleurisy (eight cases, average 0.74 per cent., variations from 1.07 to 0.55 per cent.), erysipelas (four cases, average 0.72 per cent., variations from 0.87 to 0.58 per cent.), suppurative infections (sixteen cases, average 0.68 per cent., variations from 1.01 to 0.46 per cent.), diphtheria (five cases, average 0.63 per cent., variations from 0.73 to 0.48 per cent.), gonorrheal "metastasis" (four cases, average 0.61 per cent., variations from 0.81 to 0.48 per cent.), scarlet fever (sixteen cases, average 0.60 per cent., variations from 0.83 to 0.45 per cent.), angina (ten cases, average 0.57 per cent., variations from 0.80

25. Pernicious anemia: 23 cases, average 0.26 per cent., variations from 0.37 to 0.10 per cent. Only four cases show less than 0.20 per cent. fibrin in the plasma.

to 0.46 per cent.), and bronchitis (fifteen cases, average 0.57 per cent., variations from 0.91 to 0.40 per cent.).

The increase is slighter and may keep within the upper boundaries of the normal in uncomplicated measles (two cases, average 0.42 per cent., variations from 0.42 to 0.43 per cent.), typhoid (five cases, average 0.44 per cent., variations from 0.50 to 0.36 per cent.), influenza (thirty-three cases, average 0.38 per cent., variations from 0.48 to 0.30 per cent.), "closed tuberculosis" (seven cases, average 0.36 per cent., variations from 0.43 to 0.25 per cent.), malaria (three cases, average 0.35 per cent., variations from 0.38 to 0.30 per cent.), and syphilis (six fresh cases, average 0.35 per cent., variations from 0.42 to 0.27 per cent. and six old cases, average 0.47 per cent., variations from 0.66 to 0.31 per cent.).

If one of these last named infections is complicated by one belonging to the first group, there occurs an immediate rise in the fibrin percentage.

This was demonstrated in: (1) measles with bronchitis or otitis (four cases, average 0.53 per cent., variations from 0.58 to 0.47 per cent.); (2) influenza with bronchitis (five cases, average 0.55 per cent., variations from 0.66 to 0.48 per cent.); (3) influenza with angina or otitis (three cases, average 0.58 per cent., variations from 0.60 to 0.57 per cent.); (4) influenzal pneumonia (twenty-two cases, average 0.62 per cent., variations from 0.98 to 0.36 per cent.); (5) tuberculosis of the lungs (twenty-one cases, average 0.62 per cent., variations from 1.22 to 0.33 per cent.).

Outside of the infectious diseases proper, an increased fibrin percentage in the plasma was often or always found in the following pathologic and physiologic states: (1) After intramuscular injection of sterile milk (two cases, average 0.465 per cent., variations from 0.48 to 0.45 per cent.); (2) polyarthritis chronica progressiva (ten cases, average 0.525 per cent., variations from 0.70 to 0.36 per cent.); (3) malignant tumors (excluding cancer of the liver or pancreas: twenty-six cases, average 0.55 per cent., variations from 0.81 to 0.31 per cent.); (4) nephritis (thirteen uncomplicated cases, average 0.41 per cent., variations from 0.80 to 0.27 per cent.; ten cases with fever or lung complications, average 0.62 per cent., variations from 0.89 to 0.50 per cent.); (5) pregnancy (second month, one case, average 0.33 per cent.; third month, one case, average 0.42 per cent.; fourth month, three cases, average 0.36 per cent., variations from 0.39 to 0.33 per cent.; fifth month, four cases, average 0.39 per cent., variations from 0.44 to 0.32 per cent.; sixth month, nine cases, average 0.43 per cent., variations from 0.49 to 0.32 per cent.; seventh month, ten cases, average 0.46 per cent., variations from 0.52 to 0.40 per cent.; eighth month, seven cases, average 0.57 per cent., variations from 0.83 to 0.44 per cent.; ninth

TABLE 1.—SEDIMENTATION AFTER TEN MINUTES (ABSCISSA), CELL-VOLUME PERCENTAGE (ORDINATE) AND FIBRIN PERCENTAGE IN PLASMA (RUBRIC NUMERALS) IN FIVE HUNDRED AND FORTY-TWO BLOOD SPECIMENS. THE RUBRIC NUMERALS ARE MEAN VALUES OF THE SPECIMENS, WHICH SHOW AN EQUAL SEDIMENTATION AND A NEARLY EQUAL CELL-VOLUME*

	Sedimentation after 10 Minutes in Centimeters																			
	0	Trace	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.1	1.2	1.3	1.4	1.5	1.6	1.7	1.8
0-10				0.10	0.96	0.92	0.92	0.92	0.92	0.92	0.92	0.92	0.92	0.92	0.92	0.92	0.92	0.92	0.92	0.92
11-15				0.24	0.28	0.28	0.28	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31
16-20				0.25	0.28	0.28	0.31	0.40	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38
21-25		0.20	0.27	0.28	0.27	0.27	0.27	0.43	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48
26-30		0.10	0.27	0.28	0.27	0.27	0.27	0.43	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48
31-35			0.29	0.38	0.47	0.53	0.59	0.58	0.79	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78
36-40		0.33	0.47	0.51	0.53	0.59	0.58	0.79	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78
41-45		0.34	0.52	0.59	0.58	0.55	0.51	0.70	0.86	0.86	0.86	0.86	0.86	0.86	0.86	0.86	0.86	0.86	0.86	0.86
46-50	0.24	0.35	0.49				0.73								0.97	1.22				
51-55	0.25	0.34																		
56-60	0.28																			
61-65	0.30																			
66-70																				

* In considering this table one must remember that a sedimentation <0.1 cm. after ten minutes may be met with in specimens with a normal cell volume, but a slightly increased fibrin percentage. In severe anemia the borderline between plasma and corpuscles is not clearly defined, so that the reading must be somewhat arbitrary.

month, seven cases, average 0.49 per cent., variations from 0.56 to 0.45 per cent.; tenth month, eighteen cases, average 0.55 per cent., variations from 0.74 to 0.39 per cent.); (6) catarrhal jaundice (four cases, average 0.38 per cent., variations from 0.54 to 0.29 per cent.); (7) cholelithiasis (two cases, average 0.415 per cent., variations from 0.42 to 0.41 per cent.).

These "hyperinoses" may in some cases be explained by complicating infections.

This holds true in connection with the increased fibrin percentages found in many cases of decompensated heart disease and some cases of nephritis with stasis or edema of the lungs. Sometimes, however, the stasis in the liver counteracts the influence of the infection in the lungs, making the fibrin percentage normal or even decreased.

TABLE 2.—INFLUENCE OF VARIATIONS IN FIBRIN PERCENTAGE AND CELL VOLUME

Diagnosis	Date	Sedimentation in 10 Minutes, Cm.	Crusta Phlogistica	Fibrin in Plasma, per Cent.	Cell-Volume, per Cent.
1. Simple anemia	1/ 6	0.2	+	0.32	21
	1/17	0.2	Traces	0.29	21
	2/10	0.1	+	0.32	21
	2/24	0.1	+	0.29	25.5
	3/ 9	0.1	—	0.26	33
	3/23	Traces	—	0.30	39
	3/30	Traces	—	0.31	41
2. Rheumatic fever, anemia	2/ 1	1.6	++	0.94	34
	2/14	0.3	+	0.45	34
	2/21	0.2	+	0.43	37
	2/28	Traces	—	0.35	35.5
	3/ 6	0.1	—	0.32	38

The hyperinosis found in cases of malignant tumors may partly be explained by infection of ulcerated surfaces, and, in fact, one case with a closed, small cancer in the vertebral column showed no absolute increase. Still, there can be no doubt that the tumor itself exerts some influence in the same direction.

Icterus per stasis in cases of cancer of the pancreas does not prevent the hyperinosis (four cases, average 0.63 per cent., variations from 0.97 to 0.50 per cent.). In secondary, diffuse cancer of the liver the fibrin percentage in plasma may rather often, especially in the last stages, be found normal or even decreased (seven cases, average 0.47 per cent., variations from 0.72 to 0.27 per cent. [first examinations!]).

From the beginning of normal pregnancy²⁶ there occurs a tendency to hyperinosis, which however does not always pass beyond the upper boundary of normal before in the fifth or sixth month.

26. At the same time there is a more or less pronounced tendency to anemia.

There is no special increase in cases of eclampsia, in which, however, the cell volume is markedly higher than in normal pregnant women (three cases, average 0.57 per cent., variations from 0.70 to 0.48 per cent.).

The sedimentation of the corpuscles was studied on 542 specimens of citrated blood.

The result of this series (Table 1) was that a sedimentation of 0.1 cm. or more after ten minutes occurred only: (1) when the fibrin percentage in plasma was higher than normal, or (2) when the cell volume was lower than normal.

The effect of variations in these factors is shown in Table 2.

In order to demonstrate the effects of the finer changes in the fibrin percentage, a longer time of observation is necessary, but this has only been done in a few cases, which, however, showed that the sedimentation in normal blood was regulated by the same laws.

TABLE 3.—CELL VOLUME, FIBRIN PERCENTAGE AND SEDIMENTATION AFTER ONE HOUR IN TEN INDIVIDUALS OF EACH SEX

Number	Cell-Volume, per Cent.	Fibrin Percentage in Plasma	Sedimentation after 1 Hour	Number	Cell-Volume, per Cent.	Fibrin Percentage in Plasma	Sedimentation after 1 Hour
1	38	0.37	1.3	1	49	0.37	0.5
2	40	0.33	0.8	2	49	0.35	0.4
3	38	0.29	0.6	3	46	0.33	0.3
4	43	0.31	0.6	4	49	0.31	0.1
5	45	0.35	0.5	5	46	0.24	0.1
6	40	0.26	0.5	6	49	0.27	<0.1
7	30	0.27	0.4	7	48	0.24	<0.1
8	42	0.24	0.4	8	45	0.21	<0.1
9	43	0.27	0.4	9	46	0.225	<0.1
10	46	0.32	0.2	10	49	0.23	<0.1
Average	41.5	0.30	0.6	..	48	0.27	<0.2

After ten minutes one may observe, that the plasma layer formed is broader in women's than in men's blood, where it may be missing altogether.

An observation of citrated blood from ten normal women and the same number of men after one hour has given the results shown in Table 3. Women to the left, men to the right side.

From Table 3 it appears that the difference between the sedimentation in the blood of men and women is very pronounced, and that the difference in fibrin percentage will only partly explain this.

The question now is, whether the difference in the average cell volume can explain the different sedimentation found in the two sexes.

To decide this I made the following experiments: 20 cm.³ of citrated blood is taken from a normal woman, and three Oluf Thomsen tubes are each filled with exactly 5 c.c. of this.

The two specimens (1 and 2) are left to stand for one hour, and the sedimentation in centimeters is noted.

The third specimen (3) is centrifuged for one and one-half hours. The cell volume and the fibrin percentage in plasma is determined.

To Specimen 2 a little of the precipitate from Specimen 3 is added and a corresponding quantity of the plasma is taken off.

Specimens 1 and 2b are then shaken energetically, and left to stand for another hour, the sedimentation again being noted in both.

Both specimens (1 and 2b) are then centrifuged and the cell volume in both is determined.

TABLE 4.—SEDIMENTATION AFTER ONE HOUR IN CITRATED BLOOD BEFORE AND AFTER AN ARTIFICIAL INCREASE OF THE CELL VOLUME: TWO CASES

	Sedimenta- tion after 1 Hour	Cell Volume, per Cent.	Fibrin Percentage in Plasma
Experiment A:			
1.....	0.9	40	0.34
2.....	0.9	40	0.34
1b.....	0.8	40	0.34
2b.....	0.3	47	0.34
Experiment B:			
1.....	0.4	43	0.27
2.....	0.4	42	0.27
1b.....	0.35	42	0.27
2b.....	<0.1	49	0.27

These two experiments (Table 4) seem to show that the principal cause of the difference in sedimentation between the blood of the sexes must be sought in the different averages of the cell volume.

There can be no doubt that there is a considerable experimental error in the determination of the velocity of sedimentation by this method. However, a series of double specimens and double specimens with variations in the amount of blood added to the citrate gave nearly uniform values.

A more serious error is introduced by the variations in the room temperature. This is shown in Table 5 on two double specimens which are kept respectively at 20 and 28 Celsius.

TABLE 5.—SEDIMENTATION IN TWO PATHOLOGIC DOUBLE SPECIMENS KEPT AT DIFFERENT TEMPERATURES *

Sedimentation in Specimen I Kept at 20 Celsius, Cml.	Sedimentation in Specimen II Kept at 28 Celsius, Cml.
1.5	2.0
0.3	0.5

* The sedimentation increases with the surrounding temperature till 30 Celsius.

It may also be questioned whether it is the number of cells or the cell volume which influences the velocity of sedimentation.

This was decided by determining the sedimentation on two specimens, respectively, from a case of simple and a case of pernicious anemia (Table 6) with the same cell volume and nearly the same fibrin percentage.

In order to demonstrate more directly that the sedimentation depends on the cell volume and the fibrin (fibrinogen) percentage in plasma, I have carried out a series of special experiments.

The sedimentation was measured in the same (Oluf Thomsen) glasses and in the same way as previously.

In these experiments measured quantities of corpuscles were suspended in various organic and inorganic liquids.

These corpuscles and liquids were prepared in the following way:

(1) *Washed Corpuscles*.—From 5 to 25 cm. of blood is defibrinated, the corpuscles are centrifuged down and the serum is pipetted off. The corpuscles then are washed in 0.9 per cent. sodium chlorid and thrown down several times. The last centrifuging is specially energetic. The corpuscular mass is made homogenous by shaking.

(2) *Citrated Serum*.—Nine parts of venous blood is defibrinated. One then adds one part of a 3 per cent. citrate solution, centrifuges and removes the citrated serum.

(3) *Citrated Plasma*.—This is prepared by centrifugalization for one and one-half hours of nine parts of blood + one part of a 3 per cent. citrate solution. All cellular elements are thrown down.

TABLE 6.—THE SEDIMENTATION DEPENDING ON THE CELL VOLUME

Diagnosis	Erythrocytes, Millions per MM.	Fibrin Percentage in Plasma	Cell Volume per Cent.	Sedimentation in 10 Minutes
Anemia simplex.....	3.06	0.30	24	0.3
Anemia perniciosa.....	1.73	0.29	24	0.4

(4) *Fibrinogen-Free Plasma*.—This is prepared by heating citrated plasma to 60 C. in ten minutes. The precipitated fibrinogen is thrown down by centrifugalization.

(5) *Pure Fibrinogen*.²⁷—This is prepared by mixing equal volumes of citrated plasma and saturated sodium chlorid solution. The precipitated fibrinogen is redissolved in 0.9 per cent. sodium chlorid. The process is repeated three or four times. The fibrinogen content of the final solution is determined by adding fresh serum to a few c.c. The solution is dialyzed over night against 0.9 per cent. sodium chlorid to free it of its surplus of chlorids which prevent the agglutination.

(6) *Physiological Sodium Chlorid Solution*.—0.9 per cent. sodium chlorid.

The questions to be solved by this series of experiments are the following:

(1) *Is the rapid sedimentation in pathologic blood specimen due to properties of the corpuscles or the plasma?*

27. The process is rather delicate and possibly newer methods may give better results (Johns Hopkins Hosp. Bull., 1921, loco incerto).

The experiment shown in Table 7 seems to show that the influence of the plasma is prevalent, though the sedimentation is slower and only speeds up later, when normal corpuscles are suspended in pathologic plasma.

TABLE 7.—WASHED CORPUSCLES FROM A PATIENT WITH TUBERCULOSIS PULMONUM (FIBRIN PERCENTAGE IN PLASMA 0.59) AND FROM A NORMAL INDIVIDUAL. CITRATED PLASMA FROM THE SAME PERSONS

Time After Shaking	1.5 Cm. ³ Tuberculosis Corpuscles + 3.5 Cm. ³ Tuberculosis Plasma	1.5 Cm. ³ Normal Corpuscles + 3.5 Cm. ³ Tuberculosis Plasma	1.5 Cm. ³ Tuberculosis Corpuscles + 3.5 Cm. ³ Normal Plasma	1.5 Cm. ³ Normal Corpuscles + 3.5 Cm. ³ Normal Plasma
	Sedimentation	Sedimentation	Sedimentation	Sedimentation
5 min.	0.2	Traces	Traces	Traces
10 min.	0.8	0.1	0.1	0.1
15 min.	1.7	0.3	0.2	0.3
20 min.	2.3	1.4	0.4	0.4
25 min.	2.8	2.3	0.5	0.6
30 min.	3.0	2.6	0.7	0.8

On the other hand, there is no considerable difference in sedimentation when respectively "normal" and "pathologic" corpuscles are suspended in normal plasma.

(2) *Is the velocity of sedimentation altered when the corpuscles are suspended in citrated serum instead of citrated plasma from the same individual?*

The experiment described in Table 8 shows that the sedimentation is enormously decreased when citrated serum is used instead of citrated plasma.²⁸

TABLE 8.—WASHED CORPUSCLES FROM A PATIENT WITH RHEUMATIC FEVER (FIBRIN PERCENTAGE IN PLASMA 0.90), CITRATED PLASMA AND CITRATED SERUM FROM THE SAME PATIENT

Time After Shaking	2 Cm. ³ Corpuscles + 3 Cm. ³ Citrated Serum	2 Cm. ³ Corpuscles + 3 Cm. ³ Citrated Plasma	1 Cm. ³ Corpuscles + 4 Cm. ³ Citrated Serum	1 Cm. ³ Corpuscles + 4 Cm. ³ Citrated Plasma
	Sedimentation	Sedimentation	Sedimentation	Sedimentation
10 min.	0	0.2	0.2	2.0
15 min.	Traces	0.8	0.3	3.2
20 min.	Traces	1.4	0.6	3.5
25 min.	0.1	1.9	0.9	3.6
30 min.	0.1	2.2	1.2	3.7

The experiment is carried out twice, respectively with 1 and 2 cm.³ corpuscles. It also shows that a lowering of the cell volume increases the sedimentation both in plasma and in serum.

28. As the platelets and all other cells are thrown down by the preparation of the plasma, their absence in the serum cannot be the cause of the difference between these two mediums of suspension.

(3) *Is the sedimentation altered, when the corpuscles are suspended in "fibrinogen free" plasma instead of citrated plasma from the same individual?*

The experiment shown in Table 9 shows that the sedimentation, *ceteris paribus*, is decreased considerably when the corpuscles are suspended in a plasma deprived of its fibrinogen by heat coagulation.

TABLE 9.—WASHED CORPUSCLES FROM A PATIENT SUFFERING FROM NEPHRITIS (FIBRIN PERCENTAGE IN PLASMA 0.48), CITRATED PLASMA AND "FIBRINOGEN-FREE" PLASMA FROM THE SAME PATIENT

Time After Shaking	2 Cm. ³ Corpuscles + 3 Cm. ³ Fibrinogen-free Plasma	2 Cm. ³ Corpuscles + 3 Cm. ³ Citrated Plasma	1 Cm. ³ Corpuscles + 4 Cm. ³ Fibrinogen-free Plasma	1 Cm. ³ Corpuscles + 4 Cm. ³ Citrated Plasma
	Sedimentation	Sedimentation	Sedimentation	Sedimentation
10 min.	Traces	0.1	0.5	1.0
15 min.	0.1	0.3	0.9	1.7
20 min.	0.2	0.5	1.3	2.4
25 min.	0.2	0.8	1.7	3.0
30 min.	0.3	1.0	1.9	3.2

As in the previous experiment, this also shows that a lowering of the cell volume increases the sedimentation both in ordinary and in fibrinogen free plasma.

(4) *Is the sedimentation different when corpuscles are suspended respectively in physiologic sodium chlorid solution and in citrated serum?*

TABLE 10.—WASHED CORPUSCLES AND CITRATED SERUM FROM THE SAME PERSON WITH PRESUMABLY NORMAL FIBRIN PERCENTAGE. PHYSIOLOGIC SODIUM CHLORID SOLUTION *

Time After Shaking	2 Cm. ³ Corpuscles + 3 Cm. ³ 0.9% NaCl	2 Cm. ³ Corpuscles + 3 Cm. ³ Citrated Serum	1 Cm. ³ Corpuscles + 4 Cm. ³ 0.9% NaCl	1 Cm. ³ Corpuscles + 4 Cm. ³ Citrated Serum
	Sedimentation	Sedimentation	Sedimentation	Sedimentation
10 min.	Traces	Traces	Traces	<0.1
15 min.	Traces	<0.1	<0.1	0.1
20 min.	Traces	<0.1	<0.1	0.1
25 min.	Traces	<0.1	<0.1	0.2
30 min.	Traces	<.01	<0.1	0.3
24 hrs.	0.5	1.9	2.8	3.6

* In this and the following tables I have distinguished between traces of sedimentation and a sedimentation <0.1, which latter means that the lower meniscus of the plasma (serum) surface is visible.

The experiment shown in Table 10 shows that the sedimentation, *ceteris paribus*, is more rapid in citrated serum than in physiologic sodium chlorid solution. This, however, may partly be due to the action of the sodium chlorid. As in the previous experiments, this has been done with different cell volumes, which shows that a lowering of the cell volume increases the sedimentation both in citrated serum and in physiologic sodium chlorid solution.

The specimens have been observed for a longer period, since the sedimentation is very slow.

(5) *Do the corpuscles settle more quickly in pathologic than in normal citrated serum?*

The experiment shown in Table 11 tends to show that the corpuscles settle a little more quickly in citrated serum from cases with hyperinosis than serum from normal individuals.

(6) *Will the addition of fibrinogen to a liquid cause a more rapid sedimentation of the corpuscles suspended therein?*

The addition of "pure" fibrinogen to a 0.9 per cent. sodium chlorid solution at first caused a distinct decrease in the sedimentation. The fibrinogen contained a large amount of sodium chlorid, which in concentrations above 1 per cent. (compare: Schwyzer¹⁷) prevented the agglutination.

TABLE 11.—WASHED CORPUSCLES FROM A NORMAL PERSON (FIBRIN PERCENTAGE IN CITRATED PLASMA 0.21), CITRATED SERUM FROM THE SAME PERSON AND FROM A PATIENT WITH PLEURISY (FIBRIN PERCENTAGE IN CITRATED PLASMA 0.51)

Time After Shaking	2 Cm. ³ Normal Corpuscles + 3 Cm. ³ Normal Citrated Serum	2 Cm. ³ Normal Corpuscles + 3 Cm. ³ Pleurisy Citrated Serum
	Sedimentation	Sedimentation
10 min.	Traces	Traces
20 min.	Traces	Traces
30 min.	Traces	<0.1
40 min.	Traces	<0.1
50 min.	<0.1	<0.1
60 min.	<0.1	0.1

When the salt was eliminated by dialysis, the results as shown in Tables 12 and 13 were quite clear and showed that even a low fibrinogen content will increase the sedimentation in physiologic sodium chlorid solution.

Microscopy of citrated blood has shown that the corpuscles on standing are agglutinated and then settle more quickly. This phenomenon is more pronounced the more fibrinogen the plasma contains.

About these "auto-agglutinins," which cause the agglutination of the corpuscles, we know the following:

(1) Their action varies proportionally with the amount of fibrinogen in the plasma.

(2) Their action is extremely weakened, when the fibrinogen is eliminated by spontaneous coagulation.

(3) Their action is also weakened when the fibrinogen is eliminated by heat coagulation at 60 C.

(4) The pure fibrinogen prepared by repeated precipitation with sodium chlorid shows—when freed of sodium chlorid—the agglutinating action of these bodies.

(5) The serum shows a slight agglutinating action which is stronger in serum whose plasma shows hyperinosis.

Since we know these "agglutinins" only by their action, and this is intimately connected with the fibrinogen, one may fancy that it is the fibrinogen and in a lesser degree other plasma proteids which possess these properties.

TABLE 12.—WASHED CORPUSCLES AND PURE FIBRINOGEN FROM A NORMAL INDIVIDUAL. THE FIBRINOGEN IS DISSOLVED IN 0.9 PER CENT. SODIUM CHLORID (0.19 PER CENT.)

Time After Shaking	1.5 Cm. ³ Corpuscles + 3.5 Cm. ³ 0.9% NaCl	1.5 Cm. ³ Corpuscles + 3.5 Cm. ³ Fibrinogen Solution
	Sedimentation	Sedimentation
5 min.	0	Traces
10 min.	Traces	<0.1
15 min.	Traces	<0.1
20 min.	Traces	<0.1
25 min.	<0.1	0.1
30 min.	<0.1	0.1

Whether the action of these bodies in the last instance depends on their influence on the electrical charge of the corpuscles is not shown by my experiments.

The results of the examination for crusta formation has shown that only in one out of twenty-five women and in no normal man were there found traces of a "buffy coat" on the blood.

The woman mentioned had a rather high normal fibrin percentage and a cell volume at the very lowest limit of the normal.

TABLE 13.—WASHED CORPUSCLES AND FIBRINOGEN FROM A NORMAL INDIVIDUAL. THE FIBRINOGEN IS DISSOLVED IN 0.9 PER CENT. SODIUM CHLORID (0.22 PER CENT.). THE EXPERIMENT HAS BEEN MADE ON DOUBLE SPECIMENS

Time After Shaking	1.5 Cm. ³ Corpuscles + 3.5 Cm. ³ 0.9% NaCl	1.5 Cm. ³ Corpuscles + 3.5 Cm. ³ 0.9% NaCl	1.5 Cm. ³ Corpuscles + 3.5 Cm. ³ Fibrino- gen Solution	1.5 Cm. ³ Corpuscles + 3.5 Cm. ³ Fibrino- gen Solution
	Sedimentation	Sedimentation	Sedimentation	Sedimentation
5 min.	0	0	Traces	Traces
10 min.	Traces	Traces	Traces	Traces
15 min.	Traces	Traces	<0.1	<0.1
20 min.	Traces	Traces	<0.1	<0.1
25 min.	Traces	Traces	<0.1	<0.1
30 min.	Traces	Traces	0.1	0.1
60 min.	<0.1	<0.1	0.2	0.2
Later	0.1	0.1	0.5	0.5

The clinical investigations seemed to show that a crusta phlogistica is formed under two circumstances: (1) when the sedimentation of the corpuscles is more rapid than usual, and (2) when the coagulation time is longer than usual.

As mentioned above, the velocity of sedimentation is increased when the cell volume is low (anemia) or the fibrin (fibrinogen) content of the plasma is increased (hyperinosis).

The velocity of sedimentation also is increased when the temperature of the blood is higher.

A determination of the coagulation time of recalcinated plasma (Gram²⁴) has shown that the changes in this value correspond well with the coagulation time of "natural" blood. A possible exception may be found in some cases of icterus.

A lengthening of the clotting time may be found under two circumstances: (a) as a result of an alteration in the composition of the blood, and (b) as a result of the conditions under which the blood is taken and kept.

In hemophilia and in diseases which are accompanied by thrombopenia (pernicious anemia, lymphatic leukemia, purpura idiopathica and influenzal pneumonia (Gram²⁴)) the coagulation time may be longer than usual (i. e. > 6 minutes).

If the blood is kept in a paraffinated glass or at low temperature the clotting time is prolonged.

TABLE 14.—THE PROPORTIONAL DIVISION OF THE CASES ACCORDING TO THE SIZE OF THE CRUSTA WITHIN THE DIFFERENT DEGREES OF SEDIMENTATION. FIVE HUNDRED AND TWENTY-SIX CASES

Sedimentation in 10 Minutes Cm.	Crusta Phlogistica			
	— Per Cent.	Traces Per Cent.	± Per Cent.	++ Per Cent.
0	100	0	0	0
Traces	89	8	3	0
0.1	25	37	38	0
0.2	3	20	77	0
0.3	0	6	91	3
0.4	0	13	83	4
0.5	0	6	76	18
0.6 and more	0	7	40	53

A consideration of my material shows with great certainty, that the first factor (sedimentation) is the most frequent and important cause for the formation of a buffy coat.

If we arrange the correlated values for sedimentation and crusta formation in classes, we get the results shown in Table 14.

Table 14 shows that in some cases no crusta is met with, though the sedimentation is clearly increased. This, however, happens only when the increase is relatively slight (from 0.1 to 0.2 cm. in ten minutes).

This may be due to circumstances connected with the taking and the conservation of the blood. A bad venepuncture or unclean glasses may shorten the coagulation time of the blood, and thus prevent the crusta formation.

Variations in the room temperature will be compensated partly, since an increase of the temperature makes the sedimentation more rapid, but on the other hand shortens the clotting time.

According to Table 14 one finds some cases with only traces (i. e., < 0.1 cm.) of sedimentation in ten minutes, but with a more or

less pronounced crusta. These variations may partly be explained by circumstances connected with the taking and conservation of the blood.²⁹

If we consider the diagnosis in the last named cases we find, however, that nearly all of them may be placed in one of two groups: (1) Patients with a high normal or slightly increased fibrin percentage in plasma or a low normal or slightly decreased cell volume; (2) patients with thrombopenia (pupura, pernicious anemia, influenza) or hemophilia.

A crusta phlogistica in venous blood, when 1 cm.³ is kept at room temperature in a test tube with a diameter of 9 or 10 mm. is nearly always a pathologic phenomenon, which may be due to anemia, hyperinosis, thrombopenia or hemophilia.

TABLE 15.—SEDIMENTATION AFTER ONE HOUR IN SIX NORMAL AND PATHOLOGIC SPECIMENS OF CITRATED BLOOD

Number (from the left)	I	II	III	IV	V*	VI
Diagnosis	Normal man	Normal man	Normal woman	Nephritis	Pernicious anemia	Pneumonia
Sedimentation after 1 hour, Cm.	<0.1	0.2	0.4	1.1	2.6	2.8
Cell-volume per Cent.	49	51	42	47	10	42
Fibrin, per Cent. in plasma	0.29	0.34	0.27	0.44	0.27	1.31

* Owing to the intense yellow color of the plasma the photograph does not show any difference between plasma and corpuscles in this case (nr. V). The border-line however was clearly visible to the naked eye and is marked by an arrow on the figure.

SUMMARY

1. In a large number (many hundreds) of cases the fibrin percentage in plasma (and blood), the cell volume percentage and sometimes the coagulation time has been determined.

29. A small series of experiments has proved that the use of a paraffinated glass may cause crusta formation in the blood of some normal women and even a few men. The best results are obtained when the needle also is paraffinated.

2. The fibrin content of the plasma is found to be increased in nearly all infectious diseases, in cancer, nephritis, pregnancy, polyarthritis and after infections of sterile milk. These alterations may all be considered as the effect of the introduction into the circulation of proteids foreign to the blood. A low fibrin percentage in plasma is only found in some, but not nearly all, severe degenerations of the liver.

3. The cell volume, besides in the diseases of the blood proper, is frequently found low, in certain infections, in cancer, pregnancy and polyarthritis.

4. In specimens of citrated blood, the sedimentation has been studied by an observation of the plasma layer formed after ten minutes. Under the conditions described, the formation of a plasma layer of 0.1 cm. or more must be considered distinctly pathologic and is found only in cases showing anemia or hyperinosis.

A clearer analysis of the influence of the fibrin and cell volume percentages may be made by observing the plasma layer formed after one hour. The difference in sedimentation as between normal men and women is shown to depend mainly on the different cell-volumes, less on differences in the fibrin percentage.

5. The formation of the *crusta phlogistica* (in pathologic cases) has been observed in 1 cm.³ of venous blood kept at room temperature in a miniature testtube. The formation of a buffy coat generally coincides with a sedimentation of 0.1 cm. or more in the citrated blood, divergences being found only in border cases and in cases with a lengthened clotting of the blood.

6. From clinical and experimental observations we reach the following conclusions: The formation of a *crusta phlogistica* is a pathologic phenomenon which depends on (a) an accelerated sedimentation, or (b) a lengthened clotting time.

The sedimentation of the corpuscles depends on: (1) the fibrin (fibrinogen) percentage in the plasma, the sedimentation being accelerated by a rise in this value, and vice versa. The fibrinogen brings this about by causing an agglutination of the corpuscles, which facilitates their sedimentation; (2) the cell volume percentage, the sedimentation being accelerated by a drop in this value, and vice versa; (3) the temperature, the sedimentation being accelerated when the temperature is higher, and vice versa.

The first two factors may counteract or assist one another, so that a knowledge of one of these, together with a determination of the velocity of sedimentation, allows an estimate of the other factor, that is, if the temperature of the surroundings is constant.

The coagulation time of the blood is found lengthened in hemophilia, and diseases which cause a thrombopenia, i. e., pernicious anemia, lymphatic leukemia, influenzal pneumonia, etc.

This last factor is not nearly so important, practically, as the velocity of sedimentation.

However, an accidental shortening of the clotting time may occasionally prevent the formation of a crusta, though the sedimentation is more rapid than normally.

The three factors mainly affecting the coagulation time are: (1) platelet count; (2) properties of the plasma (hemophilia), and (3) temperature.

7. A single specimen of 4.5 cm.³ blood taken into an Oluf Thomsen glass (graduated centrifuge tube) allows the determination of the following values: (1) platelet count (Oluf Thomsen ²²); (2) coagulation time (Gram ²⁴); (3) plasma color (Meulengracht ³⁰); (4) fibrin percentage in plasma and blood; (5) cell volume percentage; (6) velocity of sedimentation.

Another specimen of 1 cm.³ allows an observation of the crusta formation.

30. Meulengracht: Ugeskr. f. Læger **46**:1785, 1919.