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The Influence of changes in the blood fibrinogen level on the sedimentation rate of erythrocytes

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THE INFLUENCE OF CHANGES IN THE BLOOD FIBRINOGEN LEVEL ON THE SEDIMENTATION RATE OF ERYTHROCYTES.

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by

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Introduction

The behavior of drawn human blood has held the interest of the physicians at periodic intervals for about 2500 years. According to Fahraeus (6), the Greek physician-philosophers built great theories around their macroscopic observations of blood collected from cut vessels, and their conclusions held sway over the clinical practicioners for many centuries. Later scattered reports were made concerning the behavior of drawn blood by great men of medicine such as Hunter, Hayem, Sydenham, et al, and the term "buffy coat" was introduced into the literature. The "buffy coat" was studied from all aspects and from these studies grew the present day interest in blood fibrinogen levels and sedimentation rate. The modern study of these phenomena was started by Fahraeus as he searched for a test for pregnancy in 1918. Since this time, the literature has been flooded with articles concerning the clinical application and interpretation of sedimentation rate of erythrocytes and a great number of these articles state incidentally that the blood fibrinogen is the controlling factor in sedimentation rate changes, however, few of the articles offer any proof of their statement.

History

The earliest interest in the behavior of drawn human blood is shown in the writings of the Greek physician Empedocles (490-430 B.C.,) and his philosophy was further developed by Hippocrates (460-377 B.C.) and Aristoteles (384-323 B.C.). These early writers believed that all matter was composed of four basic substances which were identified by whether they were warm or cold, humid or dry. The following table shows how Empedocles identified the basic substances:

Air.....Warm and humid

Water.....Cold and humid

Fire.....Warm and dry

Earth.....Cold and dry

The human body was believed to be composed of fluids analogous to the four basic substances:

Air.....The blood (sanguis)

Water.....The phlegm (phlegma, pituita, fibrin)

Fire.....Yellow bile (cholera, flava bilis)

Earth.....Black bile (melancholia, atra bilis)

The Greeks believed that when the above fluids were mixed in the proper proportions, health prevailed and therefore concluded that pathology was based on malmixture of the fluids. These great philosophers then

reasoned that pathology on such a basis could best be treated by ridding the body of the malmixture and allowing the body a chance to bring the fluids into balance. Thus the birth of treatment by venesection occurred and enjoyed great support until the beginning of the present century, and in occasional cases of hypertensive cardiovascular disease, it is used at the present time by some physicians.

The practice of venesection gave ample material for physicians to observe the blood after it had been collected in a vessel and they believed that the four substances comprising blood could be identified by the level at which they settled out in the clotted drawn blood as shown in the following table:

Black Bile.....Formed at the bottom of the container (the dark colored lower portion of a blood cake)

Sanguis (Blood).Formed at the upper portion of blood cake (fluid from the oxygen of the air)

Yellow Bile.....Serum

Phlegm.....Present as connective substance proper in diseased blood cake (fibrin.) This substance could be gotten out of

> healthy blood by shaking and preventing clot formation. In diseased blood phlegm collected in more or less thick layer on top of the blood cake, interpreted as being the consequence of increased amount of phlegma.

An increase of any of the four fluids was held to be responsible for a diseased condition, however an increase in phlegma was considered most important in the etiology and proof of disease. The autopsy finding of fibrin coagula in the heart and great vessels is like that observed in drawn blood from a person who died of disease, however, this phenomenon was not found in persons who died by violence.

The last of the great Greek physicians, Galenus (129-201 A.D.), further developed the theory of four fluids as stated by Hippocrates to a high degree of perfection. Since the four fluid theory was adhered to, Galenus set forth the theory that the conceivable combinations which could alter the normal nature of the blood were eight in number, thus there could be only eight dyscrasias which could give rise to disease. The fall of the Greek civilization brought with it the intro-

duction of astrology and magic and a great deal of confusion existed during the middle ages; however, the emperical use of blood-letting as a treatment of disease persisted without further study as to benefit or damage caused by the process.

During the sixteenth century the concept of the four fluids became the basis of popular medicine among the common peoples, but as with common gossip the original theory of Empedocles became greatly distorted, and every person was believed to have a disposition for some particular disease depending upon the preponderance of one of the fluids. The terms applied to these persons are still in use today as phlegmatic, sanguinal, choleric, and melancholic temperaments.

The great physicians of the sixteenth century began to doubt the four fluid theory, and the great anatomist Vesalius did not believe that the fibrin coagula seen in sectioned blood vessels had been manufactured in the brain as had been set down by the Greeks. Paracelsus, without the benefit of a medical education, voiced his doubt and stated that the changes seen in the fluids were the result rather than the cause of disease. Few physicians paid heed to Paracelsus until near the end of the sixteenth century when Helmont

began to apply the chemistry of the day to the problem and found that normal blood could be made to be "sizy" by the addition of acid. Helmont's theory arose after he discovered the acid of the stomach, and he stated that alterations in the blood were secondary in relation to the diseases, which in their turn, arose from an acidity of the fluids which could be caused by alterations in gastric acidity.

The theory advanced by Helmont attracted little support, and the next important step in the development of our modern concept of sedimentation rate was the announcement of proof of the circulation of the blood by Harvey in 1628. This concept revolutionized both the physiological and pathological concepts that had existed for centuries, and most important to the subject at hand was the realization that the phlegma (fibrin) could not be manufactured in the brain and then pass to the lung where it supposedly caused blockage of the arterioles. Harvey's concept that a capillary network was required to complete the portions of the circulatory system which he could demonstrate macroscopically were conclusively proved by the feat of direct observation by Malpighi, who also demonstrated the presence of red cells in the blood. Immediately the power of agglu-

tination of blood which had been known for so many thousands of years was transferred theoretically to the newly discovered erythrocytes. The belief that blood consisted of only two constituents, namely serum and red cells, persisted far into the nineteenth century and the buffy coat (fibrin) was believed to be a newly secreted substance arising from the red blood cells.

Harvey's work was by far not completely accepted by all the medical world and the ardent followers of Hippocrates such as Sydenham advanced theories that the buffy coat was due to the inflammation of the blood. Boerhaave (5) further developed this concept and set down a standard of pathology which gauged the severity of disease. The four catagories indicating the fate of the inflammation were:

(1) In mildest cases, the obstructive matter is brought to a fluid state and is borne away in the flow of blood.

(2) If the matter cannot be released per (1) it is melted down to pus.

(3) In more severe cases of stagnation and when the fluids are sharp, gangrene ensues.

(4) A special form of inflammation appears only in the glands when the matter causing the inflammation is

especially solid and tough. It then forms a hard, callous tumor in the gland called Scirrhus, which with time may develop into cancer.

Huxham (16), one of Boerhaave's students, published an Essay on Fevers in 1739 in which the master's theories were elaborated upon. The various diseases were divided into catagories; for example, certain fevers afflicted those persons who suffered from plethora (fullbloodedness) and had an accompanying unhealthy disposition. The blood in such cases was characterized by an increase in the quantity of red blood cells, and the blood serum was supposedly increased in thickness. As this thickened type of blood passed through the vessels, Huxham states that an increased amount of friction occurred and this in turn caused an increased amount of heat to be generated resulting in driving away the thinner portions of the blood and causing the blood to become even thicker. Thus a vicious cycle was set up ultimately causing the vessels to become plugged in the process known as inflammation. Experimental proof offered for this line of thought rested upon the fact that heated blood will coagulate.

Another Boerhaave student, Gaubius, repeated the older man's theory and published the first manual in

general pathology. This text was used by Virchow far into the nineteenth century in his role as instructor in German Colleges. The great fault of the Boerhaave school was the inference of similar consistency of both flowing and coagulated blood.

John Hunter (15) laid the basis of modern methods of experimental pathology in the eighteenth century by his advancement of the theory that the "buffy coat" (fibrin) was a protective mechanism of the human body against disease. The power of life was believed by Hunter to be expressed in the size of the "blood cake" (fibrin) in drawn blood and an increased amount of contraction of the cake was believed to be similar to the contraction of a muscle and therefore an indication of an increase in life-power. Near the end of the eighteenth century and early in the nineteenth century the humoral theory of pathology faded out of view and a number of theories based upon Schelling's natural philosophy sprang to the fore. Cullen related diseases to a change in the activity of the ether which filled up the nervous system. Mesmer, Hahnemann, and Brown advanced theories that health was an equilibrium between irritability and irritation and that disease resulted from a disturbance of this equilibrium. The theories were advanced to ex-

plain diseased conditions and changes in blood.

Piorry (25), French physician and follower of Laennec, made advances in the field of physical diagnosis and solved the old debate as to whether blood changes were primary or secondary in disease in his description of a new group of diseases, "hemitis" (inflammation of the blood), however, his speculations were not all sound since he advanced the idea that the "buffy coat" displayed the classical symptoms of inflammation, i.e., dolor, rubor, tumor, and calor, just as did the involved organ. When it was pointed out to Piorry that pregnancy caused an increased amount of "buffy coat," he declared pregnancy a disease to save himself embarrassment.

Modern Advances

The theories offered to explain the chemistry and physics of the buffy coat, or specifically, what the buffy coat contained and why it collected at the top of the blood cake are numerous and many are not applicable to the discussion at hand. The scientist who built the foundation for our modern knowledge of blood chemistry is William Hewson (14), (1739-1774.) Hewson set forth the theory that the blood had three constituents, namely: blood corpuscles, serum, and coagulable lymph (fibrin), and he further demonstrated that the latter had the

property to coagulate spontaneously. He stated that the inflammatory crust or size was not a newly formed substance, but merely the coagulable lymph separated from the rest of the blood. Then he points out that there are two consequences which either separately or together could cause the white layer in drawn blood; the delayed coagulation of the blood or the increased velocity of sinking of the corpuscles. In further studies Hewson found that the sinking speed of the corpuscles is very much greater in plasma than in the serum of the same blood. He concluded from this observation that the plasma was thinner than serum and that this attenuation must be due to the differing factor; i.e., the dissolved fibrin. Hewson's use of the term "thinner" was mistaken by workers in the German schools to mean specifically lighter when it is now believed that he meant less viscous. The Boerhaave school proclaimed sizy blood to be stickier and more viscid than the normal blood.

Gulliver (11) knew of no method by which he could measure the viscosity of fluids yet proved by addition of a viscous solution of gum that the sinking velocity of erythrocytes is increased and that Hewson erred in presuming that serum was more viscous than plasma.

Nasse (22) and Gulliver (11) stated that the sinking

rapidity of cells in defibrinated blood was only a fraction of that attained in fresh blood which added further proof of error in Hewson's theory. In a handbook which laid the foundation for modern physiology published in 1833 by Johannes Muller (21), it is stated that the most important factor was the sinking-promoting character of fibrin dissolved in plasma, however, there is no further explanation of the phenomenon. Müller failed in his theory that the quantity of fibrin was the most important factor in the control of sedimentation rate to take into account the importance of agglutination of the cells.

Nasse (22) was the first of the investigators to mention that the number of cells in the blood and the degree of agglutination of those cells might have any bearing upon the rate of sedimentation. His contentions rested upon experiments in which he diluted defibrinated blood with serum which artificially reduced the quantity of corpuscles and resulted in quicker sedimentation the greater the anemia, however, Nasse stated that a rather great dilution is necessary before there is any noticeable change in settling rate.

Since both the "buffy Coat" and the settling rate of the cells had been combined into one common problem

various writers supplanted the information recorded by Hunter (15) and Nasse (22) by observations on clinical material. Jones (17) noted that there was a pronounced parallelism between the sinking rapidity of the corpuscle and its degree of agglutination and offered a technique of observing a drop of blood pressed between two slides in order to determine the agglutination. Those samples which remained diffusely florid were considered normal and those with an increase in agglutinating power were shown to have a dotted appearance. The study of agglutination of blood as a factor in the settling rate of corpuscles revealed that foreign bodies, such as a solution of gum, increased the agglutination and accelerated the settling rate. On the other hand, such substances as table salt decreased the agglutination and also reduced the settling rate of corpuscles.

The Greeks attributed the blood crust formation to an increase in one of the four fluids, namely, phlegma. The eighteenth century authorities believed the buffy material a newly formed product either from the corpuscles or the serum. During the nineteenth century, long after Hewson's (14) experiments, there was agreement that the buffy coat consisted of fibrin which was a normal constituent of blood. Thus in 1826 Scudamore (29)

attempted quantitative analysis of fibrin in sizy blood by weighing the coagulum before and after washing and drying it thus isolating and weighing the amount of fibrin contained in the coagulum. He arrived at the conclusion that the two-layer blood clot contained several times as much fibrin as a normal clot. Frenchmen Andral and Gavarret in 1840 added to the knowledge of the quantity of fibrin by measuring the amount of fibrin in the blood itself rather than the amount in a clot. Andral reported that fibrin normally amounted to 3 parts in 1000, and in sizy blood the ratio increased to between 4 and 8 parts in 1000.

Other theories on the formation of the buffy coat offered early in the nineteenth century which were rather quickly disproved included Mulder's idea that increased fat percentage was responsible. Bellingeri advanced the possibility that a reduced amount of electricity was causative, and Denis believed that the reduction of common salt served as the controlling factor.

Fahraeus (6) summarized that according to earlier work, the buffy coat arises due to: A, the retarded coagulation of the blood, or B, the increased sinking velocity of the corpuscles. A is shown to be of subordinate importance and possibly secondary to B. The in-

creased sinking velocity of the corpuscles depends on: 1, diminished quantity of the corpuscles, 2, agglutination of the corpuscles, and 3, increased quantity of fibrin. 1 is important and probably only comes into question in anemia. 2 and 3 thus remain as the two essential properties of sizy blood.

As stated before, Jones (17) offered support of the theory that fibrin was responsible for the whole phenomenon; however, Nasse (22) opposed this view and cited these facts in support of his contention: Corpuscles do sink much more slowly in defibrinated blood than in fresh blood, but they sink much more quickly in the defibrinated buffy blood than in the defibrinated normal blood; likewise in agglutination. Thus it was concluded by Nasse that the relationship between the sinking rapidity and the agglutination in healthy and unhealthy blood is maintained even in the serum after the influence of the greater or lesser quantity of fibrin has been eliminated, and that there must be other factors essential in the control of this phenomenon. Nasse also states that there were too many cases in which there was no increased quantity of fibrin but an increased amount of buffy coat was demonstrated, and this led him to state that possibly different factors were involved in

different cases.

Biernacki (1) published some information during the 1890's on his work concerning the settling rate of red corpuscles incidental to his work on a method by which the corpuscle volume could be determined by the extent of red sediment in a blood test in which the red cells had settled spontaneously. He performed his work independently of knowledge that there was any connection between his findings and the buffy coat which had been discussed in the literature for so many centuries. Biernacki's greatest contribution was to call attention to the fact that sedimentation rate of corpuscles could best be measured in blood in which coagulation had been prevented.

Schmidt, Dastre, and Arthur proved in about 1900 that not all fibrinogen was used in the formation of a blood clot and that the serum still contained a variable quantity. This added weight to Biernacki's (1) answer to Nasse (22) that the serum fibrinogen was especially great in those cases where at one and the same time a great sedimentation rate and a comparatively small quantity of fibrin were observed. Biernacki's reports show that he measured the sedimentation rate in two samples of blood in order to enhance the clinical value of

the test. In one, the coagulation was prevented by mixing natrium oxalate in solid form to the blood and the other sample was defibrinated; thus, he stated that he could also measure the volume of the fibrinogen in the plasma in the first sample and the quantity of serum fibrinogen in the defibrinated specimen.

Hayem (13) in 1882 and other French writers of his time were more systematic in their research than Biernacki (1) and listed three types of blood that were found. The differences in the bloods were shown in the character of the fibrin reticulum that appeared in the coagulation , and there was supposedly a very pronounced parallelism between the richness of the fibrin reticulum and the tendency of the red corpuscles to join. Even with this knowledge, Hayem (13), et al, never attempted to bring these two observed phenomena into relationship with each other.

In 1918, Fahraeus (5) published his first account of the use of sedimentation rate as a test for pregnancy and included the changes in rate in some diseased conditions. He used the terminology of suspension-stability of blood rather than the sedimentation rate in his studies.

In 1920, Starling (30) reported in his text that

protein substances of plasma may probably be regarded as forming a complex unit of living blood. From this the increase of fibrinogen and serum globulin may be considered as a change of general physical character of this protein of the plasma.

Again in 1921, Fahraeus (6) reported on his work with suspension-stability of blood particularly in reference to its application to the pregnant female and included in his study a monumental review of the older literature and a classic study of the factors influencing the suspension stability of blood. His conclusions were that the normal sinking values for males was 9 mm. per hour or less, and 12 mm. per hour or less for non-pregnant women. In answer to the question concerning the influence of gravity on the velocity at which particles descend in a fluid, Fahraeus states that three points are important in a system in which the particles are of higher density than the fluid medium. First, the heavier a particle is in relation to the fluid, the more rapidly it sinks. Secondly, the more viscous the fluid the more slowly does the particle sink. The third important factor is the size of the particle, in other words, the larger the particle the greater its settling rate. Stokes' Law according to Fahraeus is applicable

to blood sedimentation only under certain conditions, most importantly that the velocity of the particle is small, as is the case if: the radius of the particle is small, the viscosity of the fluid is great, and the difference between the density of the particle and the fluid is small. Cunningham added to this statement by working out mathematical coefficients which compensated for the more concentrated suspensions and thereby allowed the application of Stoke's Law to all cases.

The possible causes of the increased sinking velocity of the red corpuscles in pregnancy are listed by Fahraeus as:

(1) The specific gravity of the corpuscles is increased.

(2) The specific gravity of the plasma is diminished.

(3) The viscosity of the plasma is diminished.

(4) The size of the corpuscles is increased.

(5) The corpuscles are aggregated.

All of these possibilities were investigated by Fahraeus and he concluded that only the last (5) would be varied sufficiently to explain the differences noted in various bloods. He further states that from his work he believed that the blood proteins were the most important factor in the formation of aggregation of red

corpuscles and that fibrin was probably the most important factor in the control of this phenomenon.

Fahraeus (6) states that the plasma proteins belong to two main groups: albumin and globulin. The globulins were in turn composed of serum globulin and fibrinogen. He extracted these proteins from horse blood by use of these principles:

Serum Albumin.....Soluble in pure water and in a half-saturated solution of ammonium sulphate or a completely saturated solution of magnesium sulphate, but precipitated by a complete saturation of ammonium sulphate.

Serum Globulin....Partly soluble in water (pseudo-globulin fraction) and partly insoluble (eu-globulin fraction.) It is soluble in 27% saturated ammonium sulphate solution and a half-saturated solution of magnesium sulphate solution. It is precipitated by half-saturation with ammonium sulphate and by complete saturation with magnesium sulphate.

Fibrinogen.....Insoluble in water and precipitated by 27% saturation with ammonium sulphate and half-saturation with magnesium sulphate.

Applying these principles and the use of dialysis through parchment paper Fahraeus (6) prepared solutions

of each of the plasma proteins and measured the sedimentation of washed erythrocytes in each of these solutions with the results indicated below.

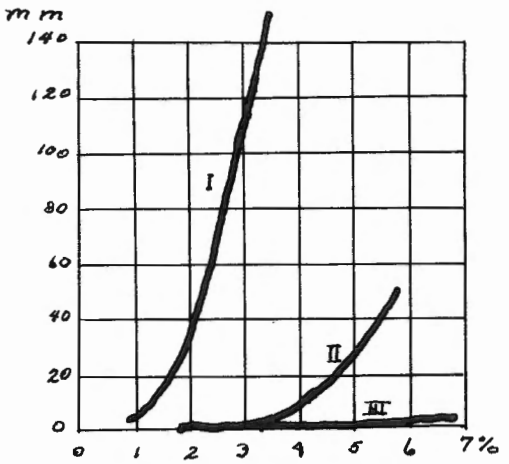

Figure I

This diagram graphically represents the sinking after 45 mins., curve I in the fibrinogen solution, II in the serum globulin and III in the serum albumin solutions.

From his many experiments Fahraeus (6) concluded that the sedimentation was consistently the most rapid in the fibrinogen solutions, less rapid in serum globulin solutions and least rapid in the serum albumin solutions and applied the term globulin increase (including both serum globulin and fibrinogen) as the most important factor in the control of sedimentation rate. As to the part played by the formation of rouleau in the sedimentation rate, Fahraeus also attributed its

control to the globulin increase.

Gram (9) stated in 1921 that the formation of buffy blood was a pathologic phenomenon which depended on two factors: an accelerated sedimentation and the lengthening of clotting time. The sedimentation of the corpuscles in turn depended on the fibrin percentage in the plasma which caused a greater agglutination of corpuscles when the fibrin was increased; the cell volume percentage which caused a reciprocal effect on the sedimentation by its changes; and temperature which caused an increased sedimentation when it became higher. In 1924 Gram (10) published a table which gave his findings on the amount of fibrin existing normally in both the blood and plasma, and the variations encountered in various blood samples were attributed to the changes in cell volume. The table below shows Gram's results.

Fibrin in Plasma and Blood

	Plasma gm. per 100cc		Blood gm. per 100cc.	
	Men	Women	Men	Women
Maximum	0.36	0.38	0.19	0.21
Minimum	0.20	0.21	0.11	0.12
Average	0.27	0.29	0.14	0.17
Subjects	25	25	25	25

Rourke and Plass (26) showed in 1928 by experiments on dogs who had decreased liver function after chloroform poisoning that there was a parallel drop in the

sedimentation rate and fibrin concentration. In 1929 Rourke and Plass (27) set forth their findings after investigating the various factors which influence the sedimentation rate of red blood cells as follows. Heparin is the ideal anticoagulant since no dilution is necessary as with citrate solution or slowing of rate as with dry materials. Aggregation of the cells in the sedimentation test is completely reversible up to twelve hours and therefore they suggested that the tests be run on the day on which the blood was drawn. Further, they found that after centrifuging a sample and remixing, the settling rate was unaffected. Other findings were that blood dilution with its own plasma increased the settling rate, and aeration, ordinary room temperature changes, changes due to food ingestion and violent exercise for a short period were all negligible in their effect.

In the same year Fahraeus (7) published his third communication in which he repeated his findings but added little new information to the explanation of the phenomenon of erythrocyte sedimentation rate.

The studies of Russell and Boyd (28) on the effect on the settling rate of red cells suspended in the lymph from the thoracic duct of dogs after intravenous adminis-

tration of peptone resulted in an increased fibrinogen concentration in the lymph and increased settling rate of red cells suspended in the lymph. Two years later Cherry (3) attributed control of sedimentation rate to red cell volume and plasma proteins. Next the results of one-hundred and ninety simultaneous measurements of fibrinogen content of the plasma and the corrected sedimentation index were made by Gilligan and Ernstene (8). These investigators concluded that there was a close correlation between the plasma fibrinogen content and the corrected sedimentation index as shown below.

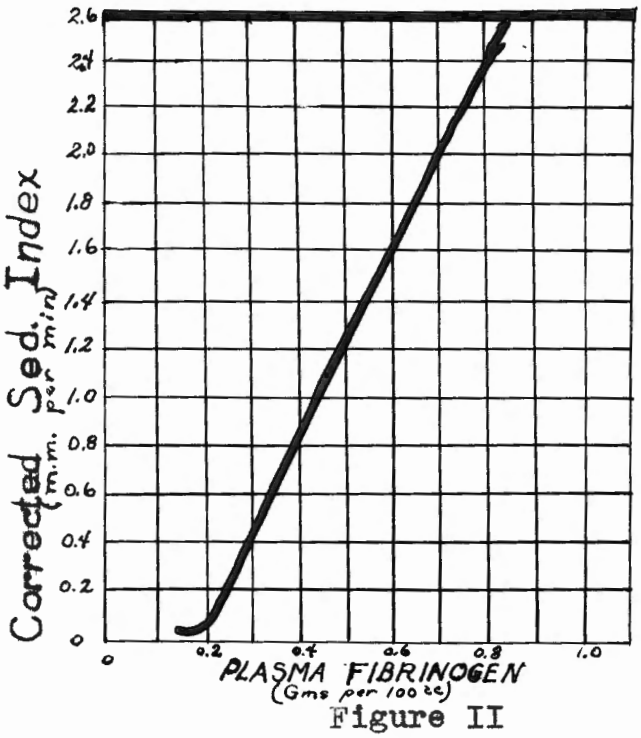

Figure II

Figure II, page 24, shows the relationship between the corrected sedimentation index and the fibrinogen content of the plasma in 190 instances, representing 39 different clinical diagnoses.

The following year Zecker and Goodell (34) stated that the sedimentation rate increased in pregnancy, malignancy, tuberculosis and acute inflammations and attributed this to the rate of tissue destruction in the body. In 1936, Lucia, (18), et al, analyzed 102 cases and found that the sedimentation rate was accelerated by increased globulin fractions of plasma and decreased by higher albumin values. They did not delve further into the study and attempt to identify the particular globulin responsible for the changes. Yardumian (33) summarized the physiochemical factors influencing sedimentation rate of red cells in an article published in 1937 which extensively covered the subject and introduced the theory that possibly all factors could be controlled except the fibrin and lipid content of the blood which he believed to be the most important controlling elements. He agreed with the earlier statements that increased fibrin level increased sedimentation but believed that increased lipid content of blood could cause the same increase and finally states that

the volume of packed cells also was a controlling factor but allowances could be made for anemia. These factors were not constant in their control of sedimentation in Yardumian's hands and he explained their control as being affected through changes in surface tension, capillary attraction or electrical charges which caused rouleau formation.

Oakley (24) found in his series of experiments in 1938 that although he could not control all the variables that were known to affect sedimentation rate, the level of fibrinogen "plays a large and probably the largest part in determining the rate of sedimentation of human blood." In the same year Ham and Curtis (12) investigated the comparative results of measuring sedimentation rates by various techniques and believed the Rourke-Ernstene method the most accurate since here they found the greatest sedimentation rate-fibrinogen level correlation. These writers believed the theory of lipid concentration proposed by Yardumian to be of little importance and stated the sedimentation phenomenon occurred because of the greater density of the cells and that the wide range of rates that were observed were due to aggregation of red cells which roughly corresponded to the fibrinogen concentration. They cast doubt that the

sedimentation rate of erythrocytes could be of any great clinical value due to the number of unknown variables that existed.

The general acceptance of the theory of plasma fibrinogen has never been complete, and in 1940, Davison (4) reported that he could find no constant relationship between the sedimentation rate and the percentage concentration of the plasma proteins. In his study of the blood of patients having a clinical diagnosis of chronic infectious arthritis Davison concludes that until all the plasma proteins are fractionated and their individual effect on sedimentation rate can be investigated, the true cause of changes in sedimentation will likely not be known.

Ira Morrison (19) published his first article on sedimentation rate in 1941 in which he warned that the tube must be perpendicular during the test period or a false increased sedimentation rate would be found. This had been known previous to this time but marks the origin of Morrison's work on the problem. In the same year Wintrobe (32) stated that there was no justification for the statement that plasma fibrinogen concentration controlled sedimentation rate and that the proper conclusion from all the previous work was that the

variation in sedimentation rate resulted from alterations in physical state of the plasma colloid system with consequent changed electrical charges in the red corpuscles and increased aggregation of the corpuscles. He also agrees with Ham and Curtis (12) that the greatest correlation in plasma fibrinogen concentration and sedimentation exists when the Rourke-Ernstene method of measuring the rate is used.

Ira Morrison (20) took the initial step in the direction shown by Davison in his work published in 1946 in which he found that yeast cells suspended in a solution of gelatin could be made to mimic red blood cells in plasma. Since the volume of blood necessary for study was not available this marked a great advance in a method to study sedimentation rates. Morrison found that there were three fractions that could be separated in the gelatin that were responsible for the sedimentation of yeast cells and in subsequent work proved this to be true of plasma fibrinogen in the sedimentation of red blood cells. Morrison states that the first fibrinogen fractions to be salted out by ammonium sulphate (16%) were those responsible for the control of sedimentation rate of red cells and of these fractions the heaviest and rubber-like fractions had a greater

control than any of the others. Thus the first step in the most modern and enlightening study of sedimentation rate of erythrocytes has been taken; however, further similar studies of the fraction of albumin, globulin and finally the various combinations of all the plasma protein fractions must be done before the exact controlling factors of sedimentation rate will be known and only then can the clinical application and interpretation of sedimentation rates be possible, accurate, and dependable.

Summary

It appears that much is left undiscovered in this field, and research concerning it should be encouraged. I find that the best account of the present problem concerning sedimentation rate was voiced adequately by Roy Nichols (23) in his review of the problem in 1942 in which he states, "The more literature that is reviewed, the more one comes to realize that the phenomenon of sedimentation is very complex, and its measurement probably does not represent the measurement of any particular constituent or property or any limited group of constituents or properties of the blood sample being considered. It represents a measurement of a balance between, ON THE ONE SIDE, the influence of ALL the consti-

tuents and properties, intrinsic or extrinsic which augment the separation of the fluid and formed elements of a particular blood sample, altered so as to allow this separation to take place, and, ON THE OTHER SIDE, the influences of all the constituents and properties which delay this separation. If this conception be reasonable, then all constituents and properties, intrinsic and extrinsic, must be causal, even though variations in some may be of more relative importance than variations in others.

"It would appear that alterations in the balance of these opposing constituents and properties may be the result of changes in the body itself (intrinsic) and changes brought about by the necessary manipulation of the blood sample (extrinsic or technical.) Since the clinical value of the test probably lies in its use as a delicate measure of the intrinsic changes in the particular sample the result of disease, then the intrinsic changes of a normal physiologic nature and the extrinsic or technical factors which may produce changes in the manifestation or the phenomenon must be enumerated, evaluated, controlled or allowed for before the measurement of sedimentation can be said to reflect pathology."

Bibliography

(1) Biernacki, see Fahraeus,R., Suspension-Stability of the Blood, Acta Med. Scandinav., 55:62, 1921.

(2) Boerhaave, see Fahraeus,R., Suspension-Stability of the Blood, Acta Med. Scandinav., 55:10, 1921.

(3) Cherry,J., Sedimentation Rate of Erythrocytes, Lab. and Clin. Med., 20:257, 1934.

(4) Davison,R., The Relationship Between Plasma Proteins and Erythrocyte Sedimentation Rates in Chronic Atrophic Arthritis, J. Lab. & Clin. Med., 25:935, June, 1940.

(5) Fahraeus, R., Uber die Ürsachen der verminderten Suspensionsstabilitat der Blutkorperchen wahrend der Schwangerschaft, Biochem. Zeitschr., 89:355, 1918.

(6) Fahraeus, R., Suspension-Stability of the Blood, Acta Med. Scandinav., 55, 1921.

(7) Fahraeus,R., Suspension-Stability of the Blood, Physiol. Rev., 9:241, 1929.

(8) Gilligan,D.R., Ernstene,A.C., The Relationship between the Erythrocyte Sedimentation Rate and the Fibrinogen Content of Plasma, Am. J. Med. Sc., 187:552, 1934.

(9) Gram,H.C., On the Causes of Variations in the Sedimentation of Corpuscles and the Formation of the Crusta Phlogistua ("Size", "Buffy Coat") on the Blood, Arch. Int. Med. 28:312, 1921.

(10) Gram,H.C., Composition and Physical Properties of Normal Human Bloods: A Compilation of Values from the Literature, Am. J. Med. Sc., 168:511, 1924.

(11) Gulliver, see Fahraeus,R., Suspension-Stability of the Blood, Acta Med. Scandinav., 55:52, 1921.

(12) Ham,T.H., Curtis,F.C., Sedimentation Rate of Erythrocytes, Med., 17:447, 1938.

(13) Hayem, see Fahraeus,R., Suspension-Stability of the Blood, Acta Med. Scandinav., 55:66, 1921.

(14) Hewson, PROPERTIES OF BLOOD, Works ed. Gulliver, London, 1846.

(15) Hunter,J., Works ed. Palmer, 3:31, 357, London, 1835.

(16) Huxham, see Fahraeus,R., Suspension-Stability of the Blood, Acta Med. Scandinav., 55:20, 1921.

(17) Jones, On the Distinction Between Healthy and Buffy Blood in minute quantities, Edinburgh Med. & Surg. J., 60:309, 1843.

(18) Lucia,S.P., et al, The Relation Between the Suspension Stability of Erythrocytes and Various Constituents of Pathologic Human Blood, Am. J. M. Sc., 192:179, 1936.

(19) Morrison,I.R., Position of the Tube in Sedimentation Rates, Am. J. Clin. Path., 11:578, 1941.

(20) Morrison,I.R., Qualitative Changes in Fibrinogen Which Influence the Erythrocyte Sedimentation Rate and the Clot Retraction Time, Am. J. Med. Sc., 211:325-331, March, 1946.

(21) Müller, see Fahraeus,R., Suspension-Stability of the Blood, Acta Med. Scandinav., 55:33, 1921.

(22) Nasse, see Fahraeus,R., Suspension-Stability of the Blood, Acta Med. Scandinav., 55:46, 1921.

(23) Nichols,R.E., A Study of the Phenomenon of Erothrocyte Sedimentation, J. Lab. Clin. Med., 27.2:1317, 1942.

(24) Oakley,W., Erythrocyte Sedimentation and the Plasma Fibrinogen, Lancet, 234:312, 1938.

(25) Piorry, see Fahraeus,R., Suspension-Stability of the Blood, Acta Med. Scandinav., 55:23, 1921.

(26) Rourke,M.D., Plass,E.D., Sedimentation and Plasma Proteins after Chloroform Administration to the Dog, Am. J. Physiol., 84:42, 1928.

Bibliography (cont'd.)

(27) Rourke,M.D., Plass,E.D., An Investigation of Various Factors Which Affect the Sedimentation Rate of the Red Blood Cells, J. Clin. Invest., 7:365, 1929.

(28) Russell,J., Boyd,T.E., Sedimentation of Erthrocytes Suspended in Lymph, Am. J. Physiol., 99:424, 1932.

(29) Scudamore, see Fahraeus,R., Suspension-Stability of the Blood, Acta Med. Scandinav., 55:54, 1921.

(30) Starling, PRINCIPLES OF HUMAN PNYSIOLOGY, p. 912, London, 1920.

(31) Sydenham, Works ed. Swan, London, 1753.

(32) Wintrobe,M.M., The Erythrocyte Sedimentation Test Am. J. Clin. Path., 11:562, 1941.

(33) Yardumian,K., Physicochemical Factors Influencing the Red Cell Sedimentation Rate, Am. J. Clin. Path., 7:105-119, March, 1937.

(34) Zecker, Goodell, The Sedimentation Rate of Erythrocytes, Am. J. Med. Sc., 169:209, 1935.