1947

Erythrocyte sedimentation rate

Clarence Zimmer
University of Nebraska Medical Center

THE ERYTHROCYTE SEDIMENTATION RATE

by

Clarence Zimmer

Senior Thesis

Presented to the College of Medicine

University of Nebraska, Omaha, 1947.

TABLE OF CONTENTS

INTRODUCTION

An increased erythrocyte sedimentation rate in the absence of disease or pregnancy is very unusual. This statement occurs numerous times in medical literature. Immediately it creates an interest in the subject, because such a test should be very helpful in detecting a diseased condition. Can this test be relied upon? Is it specific for any one disease? Can it detect the intensity of a pathological process? Is it a simple enough procedure and practical enough to be done routinely? These and many more questions immediately arise.

The purpose of this paper is to answer these questions, and to divulge the value of the erthrocyte sedimentation test. This will be done by discussing the mechanics of the test, and the factors influencing the sedimentation rate. A review of the methods used and the clinical interpretation of the test will be set forth. Finally the value of the test and its practical application will be discussed.

HISTORY

The erythrocyte sedimentation test has been used as a laboratory procedure for a mere thirty years, but the basic principle of the test, that of settling of the red blood cells, unknowningly has been used as the basis for venesection. This was the supreme method of treatment for thousands of years of medicine. To give an idea of the significant bearing of the early observations of settling of the red blood cells on the treatment of disease, it may be mentioned that the theoretical argument in favor of venesection was founded on the conception that by emptying the vessels of their contents the organism could be relieved more or less from "materia morbificans", that is, the substance of the buffy coat. We know now this coat is chiefly a fibrinous layer, and is produced by settling of the red blood cells.(27)

Fahraeus(27) points out in his historical study that in the literature of the seventeenth, eighteenth and nineteenth centuries the appearance of this fibrinous layer in the blood of diseased persons was not merely interpreted as a symptom of the disease. It was considered that the substance of which it was made up constituted the injurious matter which having aggregated

in the vascular system, represented the true cause of the disease. The formation of this layer was known under many names such as, phylogistica, pleuritica, buffy coat, size, speckhaut, couenne du sang and crusta inflammatoria. The Germans named it zerfallen blut.

The first actual scientific approach to the subject developed in 1772, when Hewsen(46) recognized that it was an increased sedimentation of red blood cells that produced the buffy coat in illness. He also noted that the sinking speed of the red corpuscles was very much greater in plasma than in serum from the same blood.

John Hunter(50), another great physician of the eighteenth century, in his observations on bloodletting of diseased persons noticed that the rapid sedimentation was associated with a granularity of the blood. This was the first time the importance of the rouleaux formation was recognized. Rouleaux themselves were not described until 1827 by Lister and Hodgkin(59). They were described as looking like piles of coins. More observations were made on the rouleaux formation in 1845 by George Guliver.(38) He recognized that the acceleration of the sinking of the red corpuscles was increased by increasing the aggregation of the corpuscles, and prevented or reversed by preventing or destroying the aggregation of the corpuscles.

The investigations during the eighteenth and nineteenth centuries were carried out only with the object of studying the buffy coat of the blood. It was not until 1921 that Fahraeus(26) discovered that the sedimentation of red blood cells was increased in pregnancy, and gave us the first practical application of the erythrocyte sedimentation phenomenon as an index of disease. He kept blood permanently fluid by addition of a anticoagulant, and using a simple technique established a range of normal limits and variations found in disease. This was a great step forward in the history of the blood sedimentation test. Since this work of Fahraeus appeared there has been volumes of literature written about the erythrocyte sedimentation test. Even with the vast amount of work that has been done on this subject, there still remains numerous points of controversy.

ROULEAUX FORMATION

When a spherical body of the dimensions of a red cell falls through a fluid such as plasma or saline, it is subject to two factors, one pulling it down and the other preventing the fall. The first factor is that of gravity. The second factor is the viscosity of the blood which opposes the fall of the particle and which increases as the velocity of the fall increases. This is the basis for the mechanics of blood sedimentation.(62)

If the red blood cells remained in the blood as small single spheres the velocity of sedimentation could be figured mathematically by Stoke's law.(71)

$$V = \frac{2\,r^2\,(S_1 - S_2)\,g}{9n}$$

V= Sedimentation rate

S_1= Specific gravity of particle

g= gravity (981 dynes)

r= radius of particle

S_2= Specific gravity of fluid

n= Viscosity

However, the red blood cells are not spheres and they do not remain discrete. In diseased blood and to a lesser degree in normal blood, the red blood cells do not settle as separate cells as is commonly supposed but as closely linked aggregates or rouleaux. The degree of

rouleaux formation is the actual index of abnormality of the blood.(20) John Hunter(50), in the eighteenth century, first noticed this phenomenon. Many different theories have been devised to explain this peculiar adhesion of cells. Fahraeus(27) thought that normally, the electric charge carried by each cell was the main repulsive force keeping them apart and that in pathological states the plasma might be altered so as to reduce this charge and allow cells to come together. A sticky substance was also proposed that would hold the cells together.

It is well agreed upon that the arrangement of the red blood cells into rouleaux and the size of the rouleaux are essentially functions of the plasma and are specific for different plasmas. The exact nature of the change in the plasma which brings about the rouleaux formation is not known.(66,17,27,71) This has been proven by washing cells that showed excessive rouleaux formation free from their plasma and suspending them in normal plasma. Here they showed no such activity. The reverse of this is also true. Normal red cells were suspended in pathological plasma. Here the cells showed abnormal fast rate of settling.(20)

Eric Ponder(71) in 1944 by suspending normal red blood cells in 5 percent solutions of 1.fibrinogen, 2.serum globulin and 3.serum albumin, found that in

fibrinogen there was an extreme degree of rouleaux formation and a rapid sedimentation rate. In serum globulin the rouleaux formation was less extensive and although less rapid than in fibrinogen was still high. In serum albumin rouleaux were not formed and the sedimentation rate was very slow.

Sellards(80) in 1908 observed that when normal human serum was heated to 60 degrees centigrade for 15 minutes or longer it developed a property of causing erythrocytes to collect into rouleaux. From this he postulated that heat increased the normal property of the serum to form rouleaux.

PLASMA PROTEINS

From the discussion on rouleaux formation it is apparent that the plasma proteins are important factors in the sedimentation of red blood cells.

Hewson(46) in 1772 first noted that erythrocyte sedimentation was greater in plasma than in serum. Since then extensive work has been done on the relationship between plasma and erythrocyte sedimentation. Nearly two hundred years later no reason can be given for this action. However, it is well known that there exists a very marked parallelism between the changes in composition of the plasma proteins and the sedimentation of erythrocytes.(25) This is substantiated by basic similar experiments of Ernstene(25), Cooper(14), Greesheimer, Johnson and Ryan(36), Ham(40), Fahraeus(27), Yardumian (99), Colburn and Kapp(13), and Ponder(71). As described under rouleaux formation, red blood cells were suspended in fibrinogen, globulin and albumin. It was shown that fibrinogen greatly increased the sedimentation rate. Globulin also increased the sedimentation rate but not so markedly, and albumin decreased it.

In 1943 Gordon and Wardley(32) by extensive experiments showed that the rate of sedimentation of the blood cells is not controlled by absolute concentration

of either total plasma proteins or by a single protein fraction. It was controlled by inhibition of one protein by another. So it was quite possible to have a high fibrinogen content and a low sedimentation rate due to an increase of the pseudoglobulin at the expense of euglobulin, while the total globulin concentration remains stationary. An increase of globoglycoid over crystal albumin can inhibit the action of the globulins without inducing any abnormality in gross protein fraction. They took protein from normal plasma and separated it into nine fractions, and the rate of fall of a 20 percent volume of erythrocytes in a 3 percent solution of the protein during one hour was found to range from 100 mm for fibrinogen to 1.5mm for albumin. The fractions were then combined in equal amounts using 39 different pairs. The action of the faster fractions, fibrinogen and euglobulin was inhibited by the slow nucleoproteins and globoglycoid. Controls were run on these experiments by using comparable natural material. The sedimentation rates were similar.

Colburn and Kapp(13) showed rheumatic fever patients who had high sedimentation rates, also had high plasma fibrinogen and serum globulin. By removing the fibrinogen from plasma in normal and rheumatic fever patients the sedimentation rate was slowed considerably.

ANEMIA

The role that anemia plays in the influence of the erythrocyte sedimentation rate provides one of the greatest controversial subjects in this discussion. In surveying the literature the opinions concerning this subject can be classified into these groups:

1. Those who firmly believe anemia accelerates the sedimentation rate and should be corrected for.
2. Those who believe that anemia affects the sedimentation rate, but that it is impossible to correct for it without destroying the value of the test.
3. Those who believe that anemia does not affect the sedimentation rate of red blood cells.

Views of all three groups will be set forth.

In the first group, or those who believe anemia affects the sedimentation rate and that it should be corrected for are: Hubbard and Geiger(49), Wintrobe and Landsberg(97), Walton(88), Gram(34), Rourke and Ernstene (75), Whitley and Hynes(94), and Bochner and Flippin(11). In general their work was done by diluting red blood cells with plasma and drawing conclusions from the results. From these results arose the idea of correction for anemia.

The first correction chart was constructed in 1928

by Gram(34). To construct this chart he altered the cell volume percentage of normal blood by the addition of normal plasma in order to obtain samples with hematocrit readings between 26 and 40 volumes percent.

In 1930 Rourke and Ernstene(75) tried to improve Gram's correction chart by the same sort of procedure but by adding only so much normal plasma to normal red cells that the hematocrit reading became 45 volumes percent. They also used heparin as an anticoagulant and warned that their chart was constructed only for heparinized blood and not blood mixed with other coagulants. It also required frequent readings.

In 1933 Wintrobe and Sandsberg(97) attempted a third correction chart. It has the advantage of only requiring reading at the end of one hour. Oxalated blood was diluted with increasing quantities of its own plasma. In this way six to nine dilutions were made of each with the blood of 13 normal men and 8 normal women. They also tested the accuracy of this test with pathological blood obtained from 31 patients suffering from varying degrees of anemia. The results were compared with the readings obtained from the chart constructed. In their conclusions they admitted that correlation although not perfect was quite high.

Three years later in 1938 Whitley and Hynes(94) also constructed a chart. In using it to correct for anemia the junction of the lines of the observed sedi-

mentation rate and the observed corpuscular volume is noted. This point will fall in one of the five zones named on the chart which indicates the approximate degree of increase in the rate. If a definite compensated figure is required, the approximate curve is followed down to the point where it cuts the 45ccm thick vertical line, which is the figure of the average corpuscular volume, and the sedimentation rate at that level is called the correction rate.

In the second group are those who believe that the sedimentation rate can not be successfully corrected for anemia. Agnor(1) running sedimentation tests on a series of 186 anemic patients obtained the following results.

Red cell count	mm 0-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	80 plus	Total
3.5-3.1	24	17	11	6	3	12	5	2	15	95
3.0-2.6	5	8	10	4	1	3	3	0	10	44
2.5-2.1	3	5	4	0	4	1	4	0	8	29
2& below	2	5	1	3	1	1	1	0	4	18
Total	34	35	26	13	9	17	13	2	37	186

In this group of 186 cases, showing definite anemia, 69 of them, or 37 percent showed a normal sedimentation rate. Even 7 of the 18 cases showing a red blood count of less than 2 million showed a normal sedimentation rate.

These figures offer a strong argument against correction for anemia when the sedimentation test is used as a routine procedure.

Gregg(37) in 1937 artifically produced anemia in rabbits by hemorrhage. He found that the degree of anemia was a factor in changes in the sedimentation rate. In acute anemia an acceleration of the sedimentation rate was nearly in direct proportion to the degree of anemia. In chronic anemia there was a gradual slowing of the sedimentation rate.

Bannick, Gregg and Guernsey(8) admit that anemia has an acceleratory effect on the sedimentation rate, but that its effects may vary with the type of anemia and the type of anticoagulant as well as the degree of anemia. They found that the sedimentation rate is in general not quite as fast in anemia as in the dilution experiments indicate, and that the various corrections for anemia tend toward over correction. They also thought that only in cases in which anemia was slight and there was a slight increase in the sedimentation rate, or in cases in which anemia is moderate or marked and there is moderate increase in the sedimentation rate that interpretation of the reading of the sedimentation rate becomes difficult. Here the sedimentation rate can be discounted or a correction for anemia made

by any method keeping in mind the possibility of the tendency toward over correction. Alston(3) is also in agreement with these last statements.

In the last group, it is believed that anemia has little to do with the phenomenon of blood sedimentation and that rapid settling is the result of the red cells forming large aggregates or rouleaux. If no aggregation takes place, sedimentation is slow no matter how marked the anemia. Cutler, Park and Herr(22), Bouton(12), Rubin and Smith(77) and Vogt(86).

ANTICOAGULANTS

In order that the settling of the red blood cells will not be hindered by formation of a clot, anticoagulants must be added to the blood. One of the most serious sources of error in the test is the formation of clots. The larger clots do not cause difficulty because they are readily seen and the test can be run over again.(65) However, small unobserable ones cause a retardation of the sedimentation rate. Therefore, the entire sample of blood must be kept fluid by anticoagulants.

Heparin is considered to produce little or no artificial change in the sedimentation rate when compared with blood from hemophiliacs.(10, 99, 76)

Ham and Curtis(42) observed in both hemophilic and non-hemophilic blood specimens, no significant alteration of the sedimentation rate when the three following anticoagulants were used.

1. Heparin 15 percent solution.
2. Dry oxalate mixture consisting of one part of potassium oxalate and 3 parts ammonium oxalate.
3. 20 percent solution of potassium oxalate.

Larger concentrations of heparin usually required to prevent coagulation always produced acceleration of the

sedimentation rate which varied directly with the concentration of heparin. Sodium citrate solution was found to retard rate because of shrinkage of cells and the dilution factor.

Belk and Wilson(10) found that single oxalates, potassium most commonly used, shrink cells and tend to retard rate. The dry mixture of ammonium and potassium oxalate in proportion of 3 to 2 cause no cell shrinkage and in most cases are comparable to heparin.

Sappington and Gillis(78) using hemophiliac blood compared untreated hemophiliac blood with hemophiliac blood to which had been added either oxalate, citrate or heparin. The results are listed in the table below.

Tube	Untreated mm/hr.	Citrated or oxalated	Heparinized mm/hr.
Cutler	13	9	16
Wintrobe	15	14	19
Westergren	14	6	21

EFFECT OF STANDING

If results of laboratory tests are not urgently needed there may be a tendency to delay running the test to make way for more pressing work. The effect of delay in settling upon the sedimentation test has marked effects. Generally a delay of 4 to 6 hours has a distinct retarding effect.(10)

Corpuscles no longer form rouleaux after standing for 7 to 9 hours at room temperature, but do so after at least 24 hours at 0 degrees centigrade.(80)

Experiments by Ham and Curtis(42) showed with heparin, 130 mg. per 100 cc of mixture, no change occurred after 6 hours of standing, however, standing 24 hours always produced extreme retardation. In using the common dry oxalate mixtures as 200 mg. per 100 cc of mixture, no change in rate after 2 hours of standing was noted, but after 3 hours there was significant slowing in 3 of 7 experiments. After 5 to 6 hours significant retardation occurred.

TEMPERATURE

According to the laws of physics the viscosity of the blood is changed by changes in temperature. This fact plays a role in the erythrocyte sedimentation rate because an increase in temperature increases the rate of the settling of the red blood cells.(76)

If the blood is at body temperature or over, the settling of the cells is accelerated. Refrigeration has the opposite effect. Between 20 and 30 degrees no significant difference was noted.(99)

Rourke and Plass(76) in a report of 11 experiments showed an average acceleration of 15 percent at 25 degrees centigrade, and 87 percnet at 38 degrees when compared to the rate observed at 20 degrees centigrade.

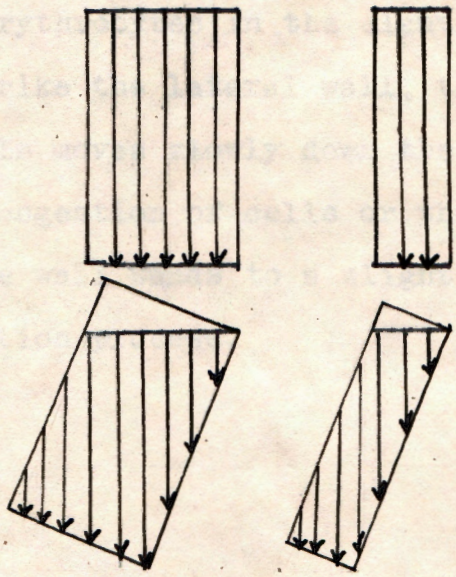
The following table was compiled by Belk and Wilson (10) in 1942. It shows the effect of temperature on the erythrocyte sedimentation rate.

Subject	4° C. mm/hr.	Room Temp. mm/hr.	37° C. mm/hr.
1. F.	1	11	31
2. F.	1	7	31
3. M.	0	5	5
4. F.	-	6	15
5. F.	-	12	24

POSITION OF TUBE

All authors agree upon this subject. The vertical position of the tube must be maintained.(10,66) An inclination of two to three percent may cause an increase in settling velocity.(97)

Inclined tubes cause increase sedimentation rates because all cells fall vertically downwards, not only from the surface of the suspension but also from the upper side of the inclined tube. The following diagram graphically shows the vertical pathways of the falling cells and the increase in the number of such pathways in an inclined tube.



When the cells fall vertically downwards, the upper side of the inclined tube should have a layer of cell free

fluid except that currents cause this layer of fluid to pass upward and to add itself to the cell free fluid above.(71)

No statement can be made regarding changes in the blood sedimentation rate due to inclination of the tube unless the diameter of the tube used is also stated.(66) For example a short tube of a large diameter might have to be tipped at 45 degrees before the relative number of vertical pathways passing through it would be doubled, where as a smaller diameter tube of the same length tipped at the same angle may thereby have its relative number of vertical pathways increased many fold. The diagram on page 19 graphically illustrates this point. The falling erythrocytes in the slanting tube will eventually strike the lateral wall, thus a concentrated stream of cells moves slowly down the dependent lateral wall. This congestion of cells or their friction against the tube wall tends to a slight extent to slow the sedimentation process.

LENGTH OF THE COLUMN OF BLOOD

There seems to be no optimum length of the blood column. The length of the blood column varies as much as a hundred percent between different methods.

The erythrocyte sedimentation rate is more rapid in longer tubes. This difference is not entirely due to the earlier packing phenomenon, but in part to a more powerful upsurge of convection currents in the short tubes.(10)

Wintrobe and Landsberg(97) in running controlled experiments on tubes of different diameter and lengths showed that the longer the tube the greater was the distance transversed by the settling corpuscles. However the distance traversed was not directly correlated with the distance to be transversed.

Fischel(29) made many tests with different quantities of blood of the same patient, emptied at the same time into sedimentation tubes of different lengths. He concluded that the percentage is a true expression of the sinking velocity. The results are listed below.

Quantity	Column	1 hour		2 hours		6 hours		24 hours	
CC	mm	mm	%	mm	%	mm	%	mm	%
2.5	119	27	23	41	34	60	50	65	54
1.5	85	2o	23	30	35	43	50	46	54
1.0	75	18	24	26	34	37	50	41	54.5

DIAMETER OF TUBE

Since the first technique for measuring blood sedimentation rate was set up there has been controversy about the internal diameter of the sedimentation tube. The trend of the literature is toward the small tube. Micro methods were for many years considered inaccurate. During the last few years, however, they have become widely used especially in Pediatric practice where capillary blood from a small puncture wound is sufficient for the test. According to extensive work carried out on the bore of the tube and its relationship to the sedimentation rate, there seems to be no difference in the sedimentation rates of tubes from 2.5 to 11mm in diameter, when the columns of blood are of equal height. Wintrobe and Landsberg(97), Walton(87), and Yardumian(99) found that below this range the readings are inaccurate. Uneven sedimentation is the reason given for the inaccurate readings. Walton(87,88) states that if the tube is of very fine diameter, the upper layer of red cells is ill defined, due to their irregular fall, and a tube of less than 2mm bore should not be used. The most constant results were obtained in tubes of 6mm bore and 6cm length. Han and Curtis(42) found a slowing of the rate in tubes of 2.5mm internal diameter. They used both 50 and 100mm length tubes. Above 2.5mm their results were the same.

By actual comparison of the micro and the macro-methods, Weisz and Taran(91) in 1943 ran simultaneous tests with the Cutler micro-method (2.5mm internal diameter) and the Wintrobe 3mm tube. Eighty one percent of the readings by the Wintrobe method in the normal group of cases are within plus or minus 2mm of the readings of the Cutler micro-method, and the remaining 19 percent fall within plus or minus 4mm. In the group having rapid sedimentation rates, 90 percent were comparable in the two methods.

Landau(57) using a micro-pipette of 1 mm diameter made simultaneous tests with Westergren (macro-method) on 300 patients. The lower values proved to correspond fairly well, where as the higher values proved to correspond fairly well, where as the higher values showed a greater difference. The Westergren method was more effective for higher values but for all practical purposes results in the micro-method were amply sufficient. Comparison between the micro-sedimentation values and the macro values in this work shows that only for the higher readings is the macro-method more sensitive.

Another extensive comparison was carried out in 1944 by McKinley and Jackson(63). Comparing the Westergren 2.5 mm with the Kato 1 mm method, 985 pairs of sedimentation rates were run from 119 rheumatic fever patients.

Both methods used the same anticoagulant, potassium oxalate, and were run simultaneously. These data were comparable from the upper limits of normal to values of 30 tp 40mm. The Westergren values having a higher range than the Kato values. The Kato method showed a more progressive change in values from 0 to 40mm, but the coefficient of correlation, 0.77, indicated a higher degree of relation between the two methods. A study of the distribution of 412 Kato values for which the corresponding Westergren values were below 15mm at 45 minutes revealed that 80 percent were 22.7mm or less, 50 percent were 15.4 or less and 30 percent were 6mm or less in 60 minutes. These values were in agreement with the normal range for the Westergren and the Kato test, 1 to 15 and 0 to 20 percent respectively.

In 1945 Peters(70) compared the values of a tube of 1.2mm with the 2.5mm Westergren. The results showed very little difference. He concluded that every one who wishes to continue to use his favorite pipette should standardize his model with the standard readings. It is probable that the Westergren "normal" values will be chosen for this purpose by an international section of a post war group of nations, because the Westergren method is by far the most commonly used method throughout the world.

Rogatz(74) compared the Landau 1 mm method with the Smith-Cutler 2.5 mm method and found that all but four out of a hundred values corresponded. In these four readings the Smith-Cutler readings were slightly increased. Previously in 1938 Rogatz had compared the Smith with the Cutler method and found satisfactory correspondence.

METHODS

Unfortunately there is no standard method used in the blood sedimentation test. A great variety of methods has confused the literature. The various techniques, although basically the same vary in the following ways.

1. Diameter of the tube.
2. Anticoagulant.
3. Height of the blood column.
4. Correction for anemia.
5. Method of timing
6. Units employed in recording the results.

These six points have been discussed in detail in previous chapters. Even though there are many controversial subjects as to the method to be used, the following are agreed upon and should be used in taking blood and setting up the test.(3)

1. Venous blood taken into a syringe with sterile technique. In micro methods capillary blood.
2. If the limb is constricted to fill a vein, the constriction should be slight or removed as the blood is withdrawn.
3. The blood and the anticoagulant must be mixed thoroughly and as soon as possible.
4. The test should be set up within a few hours of the withdrawal of blood. Described previously.

5. All the glass ware and needles used in the test must be well washed to prevent contact of antiseptics or other chemicals with the blood.

6. Hemolysis of corpuscles falsifies results.

7. Bore of the tube varies, however, these tubes must be dry and during sedimentation must be kept vertical.

8. Bubbles of air in the column of sedimenting blood must be avoided.

In order to facilitate comparison of different methods, a condensed chart of the most popular methods is given below. The Lizenmeier method , although not used now because of its method of timing, is included because of its historical interest. This was one of the earlier methods used. There are numerous other methods varying only slightly from those given below.

Author	Height column	Int. dia.	Anti-coagulant	Method timing	Normal range
Rourke & Ernstene (75)	100mm	4mm	Heparin or Dry oxalate	mm/min.	0.05-0.4 mm/min.
Wintrobe& Landsberg (97)	100mm	2.5	Dry oxalate	Fall in 1 hour	0-9 men 0-15women
Westergren (92)	200mm	2.5	3.8% Na. citrate	mm fall in 1 hr.	1-3 men 4-7 women

Author	Height column	Int. dia.	Anti-coagulant	Method timing	Normal range
Linzenmeier (42)	50mm	5mm	5% Na. citrate	Time to settle 18 mm	200 to 600 min.
Cutler (18)	50mm	5mm	3% Na. citrate	graph of curve & mm/hr.	Horizontal line 2-8mm men 2-10 women
Cutler(16) Micro-method	50mm	2.5	3% Na. citrate	Graph of curve & mm/hr.	Same as Cutler macro-method above
Smith (82)	50mm	2.5	5% Na. citrate	mm in ½ & 1 hr.	Infants&child. 1-8mm in ½ hr. 3-13 in 1 hr.
Landau (57,7)	62.5	1mm	5% Na. citrate	mm fall 1 hour	Children 1-8mm 1-5 men 1-8 women
Kato (54)	Varies 64-102	1mm or less	K. oxalate	% fall in 1 hr.	0 to 20 % in 1 hr.

GENERAL CLINICAL INTERPRETATION

In compiling this clinical survey, I will first give a general over-all picture of the use of the blood sedimentation test as a practical clinical procedure; then a more detailed account of certain diseases in which it has a more practical application.

Clinically the sedimentation test is valuable as a diagnostic and also as a prognostic procedure. The test often indicates the presence of a disease before the disease can be recognized by the usual clinical and laboratory methods. It also acts as a diagnostic gauge of the constitutional disturbance produced by a pathological process. It indicates the intensity of the disease and thus like fever or pulse rate or blood count, but more exactly, helps to complete the diagnosis in a quantitative manner.(19) As a prognostic aid, and as a guide in treatment, the sedimentation rate is often more valuable than fever, pulse rate and blood counts. The rate becomes more rapid as the disease progresses, and approaches a normal level only as the physical condition returns to normal.(42)

Before the physician can accurately interpret the test a few pertinent facts should be kept in mind. It is generally agreed that the sedimentation rate in

healthy infants is extremely low. It gradually increases to a maximum in puberty, after which it slowly decreases until the onset of old age, when it may rise again. Healthy females may have twice as fast a sedimentation rate as healthy males.(24)

It may be accepted as a working principle that a raised sedimentation rate is so unusual in health, apart from pregnancy, that it justifies a strong suspicion of an active pathological state. It may also be elevated during menstruation.(96,68)

Cutler(19) in a careful study of 5000 cases over a period of six years found that the sedimentation rate had the following limitations.

1. It does not give a diagnosis of a specific disease.
2. A normal sedimentation rate is not conclusive evidence that there is no disease, but it does show that disease if present, is producing very little if any constitutional disturbance.
3. Abnormal sedimentation reveals pathological processes that cause tissue destruction, but these include the infectious diseases and malignancy.
4. The sedimentation rate is abnormally rapid in pregnancy after the third month.

5. Five cases in over 5000 had a normal sedimentation rate and had clinically active disease.

Cutler(21) working on this subject over a period of nearly twenty years has compiled a table of diseases, grouped as determined by their sedimentation rates. Although this table may not be entirely complete it gives a working idea of the effects of different diseases on the sedimentation rate. It should prove very useful in interpretation of the sedimentation test.

I. Diseases with rapid sedimentation rates.

1. Chronic infectious diseases, such as tuberculosis and syphilis.
2. Acute infectious diseases, such as, pneumonia, septicemia, acute endocarditis and exanthemata.
3. Localized suppurations, such as pelvic inflammatory disease, suppurative mastoiditis, empyema of the gall bladder and bronchiectasis.
4. Acute intoxications, such as lead and arsenic poisoning.
5. Endocrine disturbances characterized by an increase in catabolism, such as hyperthyroidism.
6. Nephritis.
7. Malignancy.
8. Certain forms of arthritis.
9. Cardiac infarction.

II. Diseases with little or no increase in rate.

1. Uncomplicated catarrhal inflammations, such as appendicitis, simple rhinitis, colitis and catarrhal jaundice.
2. Chronic ulcerations of small extent, such as gastric or duodenal ulcer and cervical erosion.
3. Focal infections, such as abscessed teeth, pyorrhea, and tonsillitis.

III. Conditions that tend to retard sedimentation rate.

1. Anaphylaxis.
2. Polycythemia.
3. Jaundice.
4. Cyanotic conditions.
5. Infectious diseases accompanied by a leukopenia, such as typhoid and paratyphoid.
6. Pick's disease.
7. Addison's disease.
8. The sedimentation phenomenon may disappear in some cases shortly before death.

IV. Diseases not influencing sedimentation rate.

1. Functional diseases, such as various neuroses and neuroesthenia.
2. Certain nervous diseases such as, dementia praecox.

3. Metabolic diseases not characterized by increased metabolism, such as uncomplicated diabetes and essential hypertension.
4. Allergic diseases, such as asthma and hay fever.
5. Most skin diseases.
6. Simple new growths, such as fibroma, lipoma and fibromyoma.
7. Simple cysts.
8. Arrested vavular disease of the heart.
9. Malnutrition.
10. Sickle cell anemia and secondary anemia. Anemias per se do not increase sedimentation velocity.

Mathieu et al(61) in a 2000 case study found the chief value of the sedimentation rate was as a clue to previously unsuspected infection or malignancy. Along this same line a recent article by Nickols(67) stresses the importance of the erythrocyte sedimentation rate as a procedure that attracts the physician's attention to obscure or occult diseases before these diseases can be recognized by the usual laboratory methods. The following is a table of these diseases.

I. Generalized infections.
   A. Atypical rheumatic fever.
   B. Early rheumatoid spondylitis.

C. Fungus infections, such as abdominal actinomycosis.

D. Parasitic infections, such as malaria in the apyrexial stage.

II. Localized infections.

A. Abscess of the liver.

B. Tuberculosis of bone.

III. Malignant lesions.

A. Carcinoma of the pancreas.

B. Carcinoma of the liver.

C. Hypernephroma.

D. Carcinoma of the prostate gland.

E. Multiple myeloma.

IV. Lymphoblastoma.

V. Acute intoxications, such as lead poisoning.

VI. Endocrine disorders, such as Addison's disease.

VII. Vascular occlusion or inflammation, such as a mild rather silent type of coronary occlusion.

VIII. Diseases of the liver such as cirrhosis.

In the above cases, according to Nickols(67), the physical examination, the routine laboratory tests, such as leukocyte count, red cell count, hemoglobin, urinalysis, Wasserman, and X-ray of the chest were negative. The history may or may not have been of help. Often the only positive finding was an increased sedimentation rate, which called attention to the disease.

MENSTRUATION AND PREGNANCY

It is most unusual to find an increased sedimentation rate in the absence of disease or pregnancy. In reading the literature on blood sedimentation, one often encounters the previous statement. Some authors also add menstruation to the physiological causes of an increased sedimentation rate. Because these two physiological conditions may cause a misinterpretation of the sedimentation test, a more detailed study of their effect on the sedimentation rate will be given.

MENSTRUATION

There is some controversy as to the effect of menstruation on the erthrocyte sedimentation rate. Stokes(83) in his experience found that menstruation does not effect the sedimentation rate. Obermer(68) found some women consistantly show an increase in rate during the flow while others remain normal. Wintrobe (95) concluded that fluctuation of the sedimentation rate in relation to menstruation was so slight that it could be disregarded. In the literature mentioned above none of these authors have given an explanation of an increased rate if it was present, nor has any given any evidence of direct experimentation.

Greene(35) carefully questioned and examined 15

volunteers. Of these, 10 were finally chosen as above suspicion of harboring any abnormality known to influence sedimentation rate. The blood sedimentation rate of each was measured every day of a complete cycle by the Westergren standard technique. In order to test the possibility that such a short series may fail to bring out an average change, the cycle of each subject was divided up into four phases. Phase 1, which included menstruation, was from the first day of the cycle to the midpoint between this and the presumed date of ovulation; phase 2 was thence until ovulation; phase 3 covered the first half of the luteal phase; and phase 4 covered the second half of the luteal phase ending at menstruation. The average blood sedimentation rate for each period was calculated for each subject. The average reading in mm. was 6.05 for the first phase; 6.25 for the second phase; 6.25 for the third phase; 6.15 for the fourth phase. Individual variations were also slight. According to this work there is no change in the suspension stability of erythrocytes characteristic of any phase of the menstrual cycle.

PREGNANCY

From the above discussion and evidence, pregnancy alone remains the only physiological process which produces a significant acceleration of the sedimentation

rate.(83,8,96,61) All literature on the blood sedimentation rate agree that pregnancy accelerates the rate.

Obermer(68) found that in general the sedimentation rate is increased after the third month of pregnancy and continued until four weeks post partum. In conditions associated with infection, internal hemorrhage, or necrosis of tissue the rate is proportional to the extent of severity of the process.

Vogt(86) noticed a progressive increase in sedimentation rate during normal pregnancy. During puerperium the sedimentation rate returned to normal beginning after the second day post partum. The increase in the erythrocyte sedimentation rate in pregnancy is related to an increase in the plasma fibrinogen fraction of the blood.

THE SEDIMENTATION RATE IN TUBERCULOSIS

Because tuberculosis is a disease causing tissue destruction, it causes a increase in the sedimentation rate. (19,43,95)

Banyai and Anderson(9) in 1930 studies 2000 cases of tuberculosis in relation to their sedimentation rates. Because of the nonspecificity of the test it was not diagnostic. They found however, that the greatest merit of the test lies in its value as an index of the change in the general condition of the patient. It was also helpful in prescribing and controlling routine or special treatment of the tuberculous patient. In their work it was used as an aid in connection with the physicial examination for determining the patient's working capacity.

There is a marked difference in the sinking velocity between early, moderately advanced, far advanced cases and between fibrotic and ulcerative types of tuberculosis. The sedimentation test is of diagnostic value in doubtful cases of tuberculosis.(29)

Cutler(26) as early as 1926 found in a study of the erythrocyte sedimentation rate in tuberculosis that it was an aid in estimating the activity of the disease. It was more reliable than the temperature curve, pulse

rate, or gain in weight, the three major guides in the treatment of tuberculosis. He found the graphic representation of the sedimentation rates of these patients was helpful. By repeating the test at regular intervals, the true course of the disease could be graphically represented, for as the individual improved the graph approached the horizontal line, and if he became worse, the line would become more vertical.

The most recent article written on the sedimentation rate in tuberculosis is by Todd(85) He found that a patient who had a normal sedimentation rate on admission, and remained normal throughout the treatment, could be considered to have a good prognosis. It was generally found that in these patients the intrapulmonary lesion was small, and that in the majority of cases there were no physicial signs in the chest. In general it was found that the magnitude of a given rate represented the product of the amount of tissue involved, and rapidity at which the involvement was taking place. One exception to this rule was found in pleural effusion. At the unset of pleural effusion the sedimentation rate was often high, however, the readings in cases of primary pleural effusion rapidly returned to normal, unless complications occurred, such as a spread of the disease to the lung or the invasion of the fluid by secondary

organisms, and in these complications the patients continued to have a high sedimentation rate for several months.

Another interesting fact about the sedimentation rate observed by the same author, was the reaction after thoracoplasty. For instance, if the test is done daily after the operation in a number of cases it was observed that the peak rate was usually reached on the fourth day after the first stage of the operation, and on the third day after the second stage. It than began to return to its former level. In some cases the rate ran very high for some time after the fourth day. This was an indication that the patient would have a stormier course than those in whom there was a rapid return to their former levels after the peak day. Todd also found that if the test was to be of service it should be done regularly and in conjunction with other tests and x-rays. His rule was to do a sedimentation test on admission, once per month during the stay and just before leaving. It was generally thought that patients who finished their treatment at the sanatorium with a low sedimentation rate, had a better prognosis than those who finished with an increased rate. A case could not be pronounced arrested in face of a rising sedimentation rate, unless there was an obvious extraneous cause for it.

RHEUMATOID ARTHRITIS

In rheumatoid arthritis there are very few available objective standards upon which the physician may base progress or deterioration. No causative organism or its antibody has ever been isolated. Therefore a non-specific test must be employed. In this condition the blood sedimentation rate is at present the main objective measure of activity or severity of the disease.(31, 30,8,53,2)

The sedimentation rate appears to depend upon the number and size of joints affected and on the intensity of the reaction taking place in them. The increased rate is probably due to the total mass of fibrinoid necrosis in the patient, plus the effects of lysis of joint cartilage by synovial pannus and other destructive lesions of the joint.(31)

Davis(23) , in protein studies, found the globulin fraction up in rheumatoid arthritis. This also explains the increase in sedimentation rate, because in a previous chapter it was shown that the globulin fraction of the serum increased the sedimentation rate.

Beside the joint lesions, recent work by Baggenstoss and Rosenberg(6) disclosed rheumatoid heart disease in more than half of 25 cases of rheumatoid arthritis coming to necropsy. In 1944 these same authors(7) found

lesions which they regarded as being characteristically rheumatoid in type, differing in structure from heart lesions of acute rheumatism. Lesions in skeletal muscle have been found by Curtis and Pollard(15). It may be that the non-articular lesions of this type are responsible for rapid rates noted in some cases where the evidence of joint involvement seems to be inadequate for the high rate.

Gibson(31) stated that any treatment may be gauged by repeated observations of the erythrocyte sedimentation rate, and only if the disease dies out does the rate return to normal.

Hirsh(47) in a series of 23 rheumatoid arthritis patients found that in each the sedimentation rate was elevated. He also noted the uniformity of the sedimentation test over long periods of time compared to the marked variability of the leukocyte count. As the patient improved there was a tendency for sedimentation rate to decrease.

RHEUMATIC FEVER

In the past much emphasis has been laid upon the importance of nursing all children with rheumatic fever strictly recumbent for an arbitrary period of three to six months irrespective of whether or not their rheumatic state was active. This forced period of rest and convalescence can be modified if the course of the rheumatic patient is watched by means of frequent erythrocyte sedimentation determinations. The sedimentation of red blood cells in rheumatic fever patients is a sensitive index of the clinical standing of the patient.(95,89,79, 69, 63,60,55,45,25,13)

In rheumatic fever the sedimentation rate remains increased until the process has become quiescent. In this manner one can follow the gradual improvement and thus allow the patient more physical freedom. This is especially valuable in those patients who have active rheumatic involvement.(90,55)

Ernstene(25) carefully studied the erythrocyte sedimentation, plasma fibrinogen and leukocytosis as indices of rheumatic fever involvement. In all cases the sedimentation rate, plasma fibrinogen and leukocytosis were measured at frequent intervals. At the height of the illness the corrected sedimentation rate

showed a greater relative increase above normal than did the leukocyte count. Both the sedimentation rate and the leukocyte count were depressed somewhat by salicylates, but in general the sedimentation rate seemed to be less affected than the white blood count. With exacerbations of the infection in the polycyclic and the continuous types of the disease, there was usually a greater relative increase in the corrected sedimentation rate than in the leukocyte count. A general parallelism was observed throughout the course between the plasma fibrinogen and the corrected sedimentation rate. In all types of the disease the corrected sedimentation rate with few exceptions remained elevated for several days to a few weeks after the leukocyte count had returned to normal.

In all cases the infection cannot be considered arrested until the corrected sedimentation rate has become normal and remained normal.(57) The period required for return to normal after an acute episode varies from several weeks to several months.(84,45)

Often in the treatment of rheumatic fever patients the most difficult decisions arise in the group with clinically borderline activity. In these cases the test is very useful.(45) Bach and Hill(5) found that in using the sedimentation test it served as an aid in

deciding that in some cases 99.2° F. was to be regarded as the normal mouth temperature if the sedimentation rate was normal. In others, when the sedimentation rate was not increased, a heart rate of 100 was not considered due to an active carditis. In still others loss of weight and appetite were not indications of recurrent rheumatic infection if there was no increase in the sedimentation rate.

The diagnosis of subacute rheumatic fever is often made on the basis of vague aches in the legs. There are other causes than rheumatic fever for these aches. Faulty posture is often the basis of this complaint. The blood sedimentation rate is consideralbe help in sorting out these cases. With a normal figure active subacute rheumatic fever can be excluded.(79)

Numerous workers have noticed in following rheumatic patients by means of repeated sedimentation tests that after apparently normal rates in convalescence, the sedimentation rate rose sharply after salicylate therapy was discontinued. From this they concluded that salicylates have the ability to lower the sedimentation of red blood cells as well as the temperature and leukocyte count.(58,51,25)

In 1945 Homburger(48) studied the effect of sodium salicylate on sedimentation of erythrocytes in vitro. He found that sodium salicylate caused a marked reduction

of the rate particularly if it was accelerated. In fresh plasma the slowing took place at the salicylate level of about 90 milligrams per 100 millileters. No demonstrable change of plasma fibrinogen or red blood count were caused by the salicylates. From this work he concluded that the effect is inherent in the salicylate radical, because controls using sodium benzoate or sodium bicarbonate were ineffective.

Two conditions often associated with rheumatic fever are chorea and congestive heart failure. Either condition will alter the picture of the blood sedimentation rate in rheumatic fever patients and therefore, should be taken into consideration. Both conditions tend to decrease the blood sedimentation rate.(98,89,79)

MALIGNANCY

Cutler(15) in his classification of diseases according to their sedimentation rates, places malignancies in the group that has an abnormal sedimentation rate.

Nichols(67) listed carcinoma of the pancreas, carcinoma of the liver, hypernephroma, carcinoma of the prostate and multiple myeloma as diseases showing an increased sedimentation rate.

Stokes(83) in his review of the diagnostic value of the sedimentation test noted that the test could not be relied upon entirely to differentiate malignant from benign growths. It had been his observation that the sedimentation rate was usually markedly accelerated in malignancy. His experience however, with the sedimentation test as an aid in the diagnosis of malignancy was limited to bronchogenic carcinoma.

As seen in previous chapters, the rate with which the erythrocytes in citrated or oxalated blood settle is retarded if the blood is allowed to stand for several hours before the test is set up. Koster(56) in 1937 reported that in certain specimens of oxalated or citrated blood this retardation did not take place. Such exceptional changes in the sedimentation rate he found only in patients with malignant rumors and Hodgkin's disease or in patients who had been given certain drugs,

such as potassium iodide, bismuth, salvarsan, aminopyrine, lipiodol, parabrodil and tetragnost.

Koster used the following method. At fairly constant room temperature, 8 cc of venous blood were mixed with 2 cc of freshly prepared 3.8 percent sodium citrate in a graduated test tube. This constituted the store of blood from which Westergren sedimentation tubes were filled directly after the blood was drawn, then after 1,2,3,4,5,6,and 24 hours. The blood was thoroughly mixed each time before removing specimens.

He described three types of behavior in blood from patients with cancer.

Type I. The rate of sedimentation remains at the same level throughout the 24 hours, or at any rate the decrease does not amount to more than 20 percent of the initial value.

Type II. The rate of sedimentation markedly increases above the initial value in the course of the estimations and does not decrease to below the initial value.

Type III. After having risen to above the initial value the rate of sedimentation again decreases gradually often to far below the initial value.

Koster reported that the blood of 95 percent of 112

patients with cancer and 100 percent of 11 cases of Hodgkin's disease conformed to one of these three types. He did not report the frequency with which each type occured.

Feldman(28) in checking on Koster's work, made a study of 176 cases, of which 118 were proven malignancies and 55 were pathologic conditions other than malignancies. He used the same technique as Koster except for difference in mixing of the blood and for the use of the Wintrobe instead of the Westergren tube. The percentage of positive results (maintenance of the sedimentation rate) in malignant cases was 95.7 percent and of negative results was 94.6 percent in cases of non malignant diseases. Feldman also noticed that sulphanilamide and sulphapyridine influenced the sedimentation curve in a manner similar to the drugs enumerated by Koster. Neither of these authors gave a reason for this exceptional behavior of erythrocytes in these cases.

Hertz and Rinzler(44) run sedimentation tests serially according to methods of Koster and Feldman on 33 patients with proven malignancies and 67 with pathological conditions other than malignancy. The malignant cases showed 54 percent positive, and the non-malignant cases showed 72 percent negative results.

Following a slight modification of the Koster

technique Mendell and Korenberg(64) also found results which led them to conclude that the maintenance of the sedimentation rate may be used as an aid in the diagnosis of cancer, leukemia and Hodgkin's disease.

Apter, Hull and Adams(4) using a technique similar to Feldman made sedimentation rate determinations on a number of patients in whom the presence or absence of malignant disease had definitely been established. Their results demonstrate that retardation or maintenance of the initial sedimentation rate in stored blood is capricious, and depends in large part upon the temperature of the blood when the rates are determined at the temperature at which it is stored. They found when blood was stored 24 hours at 10° C., and rates determined with the blood at the same temperature, the rate remained within 20 percent of the initial rate in 4 cancer cases out of 5. It decreased more than 20 percent in a little less than half of the cases of non-malignant disease. Indicating that in an unknown case a positive test would point to the chances of cancer being present in two out of three cases, while negative tests indicate that there is only one chance in five that carcinoma is present. From this set of it was concluded that the maintenance of the sedimentation rate was not a reliable test for the presence of malignancy.

SUMMARY

The settling of the red blood cells has been noted for nearly two centuries, but the sedimentation test was first used as a laboratory procedure by Fahraeus in 1920.

The settling of the red blood cells depends upon the amount of rouleaux formation. There is no satisfactory explanation of the formation of rouleaux. An increased rouleaux formation is associated with an increase in fibrinogen and globulin in the blood. There is some evidence that the sedimentation rate is influenced by other fractions of the plasma protein. Also one fraction may inhibit the other. For example, the action of the faster fractions, fibrinogen and euglobulin is inhibited by the slower nucleoproteins and globoglycoid.

There is no satisfactory evidence in the literature as to the effects of anemia on the sedimentation rate. The greater bulk of the evidence points to the fact that anemia accelerates the blood sedimentation rate, but that corrections for anemia may be misleading.

Fifteen percent heparin used as an anticoagulant seems to produce little or no change in the sedimentation rate. Dry mixtures of potassium and ammonium oxalate,

however, appear to be very satisfactory.

In setting up the blood sedimentation test care should be taken that the tube is vertical. The test should be started within three hours of the drawing of the blood. The temperature should be between twenty and thirty degrees centigrade for the best results. Room temperature is satisfactory.

The length of the tube used varies greatly and there seems to be no optimum length.

The diameter of the tubes vary from one millimeter to six millimeters. Numerous authors object to the small capillary tubes, but much evidence has been set forth to show that they are accurate enough for practical purposes.

Numerous methods have been described. Many of them are named for men who have altered the technique slightly. The methods differ essentially in the type of coagulant, length of tube, diameter of tube and the method of timing. The greatest difference and the greatest controversy appears to be in the diameter of the tube used. Advocation of the micro method is seen more frequently in recent literature than in the older works.

Clinically the sedimentation rate is elevated in diseases causing tissue destruction. Pregnancy is the

only physiological condition where there is an increase in the sedimentation rate. There is evidence set forth to show that the sedimentation rate is a sensitive index of the pathological activity in the course of tuberculosis, rheumatic fever, rheumatoid arthritis and coronary infarction. The maintenance of an accelerated sedimentation rate in blood standing over a period of twenty four hours may, according to some authors, be diagnostic of malignancy.

From the evidence presented, the erythrocyte sedimentation test is a simple, reliable procedure that if done routinely will greatly aid the physician to discover hidden disease, or to follow the course of a disease.

BIBLIOGRAPHY

1. Agnor, E. B. : The blood sedimentation test as a diagnostic procedure; a clinical evaluation of 2063 cases.
   Ann. Int. Med. 14:774, 1940.

2. Alexander, M. E. : Clinical and experimental observations on blood sedimentation.
   Med. J. and Rec. 119:549, 1924.

3. Alston, J. M. : Methods of estimating the blood sedimentation rate.
   Practitioner 157:23, 1946.

4. Apter, L. and Adams, C. C. : Maintenance of the sedimentation rate as a test for malignant disease.
   Am. J. M. Sc. 206:168, 1943.

5. Bach, F. and Hill, N. G. : Erythrocyte sedimentation rate in rheumatic fever.
   Lancet 1:75, 1932.

6. Baggenstoss, A. H. and Rosenberg, E. F. : Cardiac lesions associated with chronic multiple arthritis.
   Arch. Int. Med. 67:241, 1941.

7. Baggenstoss, A. H. and Rosenberg, E. F. : Unusual cardiac lesions associated with chronic multiple arthritis.
   Arch. Path. 37:54, 1944.

8. Bannick, E. G. K., Gregg, R. O. and Guernsey, C. M.: The adequacy of a simple test and it practical application in clinical medicine.
   J. A. M. A. 109:1257, 1937.

9. Banyai, A. L. and Anderson, S. F. : Erythrocyte sedimentation test in tuberculosis.
   Arch. Int. Med. 46:787, 1930.

10. Belk, W. P. and Wilson, M. K. : The erythrocyte sedimentation test.
    Pennsylvania M. J. 45:1045, 1942.

11. Bochner, F. and Flippin, H. F. : A study of some of the factors influencing the sedimentation test. J. Lab. and Clin. Med. 20:583, 1935.

12. Bouton, M. S. : Erythrocyte sedimentation and anemia. J. Lab. and Clin. Med. 23:519, 1938.

13. Colburn, A. F. and Kapp, E. M. : Observations on the development of the high blood sedimentation rate in rheumatic carditis. J. Clin. Investigation 15:715, 1936.

14. Cooper, H. N. : Sedimentation rate of red blood cells. J. Lab. and Clin. Med. 11:615, 1926.

15. Curtis, A. C. and Pollard, H. M. : Unusual cardiac lesions associated with chronic multiple rheumatoid arthritis. Ann. Int. Med. 13:2265, 1940.

16. Cutler, J. W. : A finger puncture method for the blood sedimentation test. Am. J. M. Sc. 173:687,1927.

17. Cutler, J. W. : A graphic method for the sedimentation test. Am. Rev. Tuberc. 19:544, 1929.

18. Cutler, J. W. : The graphic presentation of the blood sedimentation test; a study in pulmonary tuberculosis. Am. J. M. Sc. 171:882, 1926.

19. Cutler, J. W. : The practical application of the blood sedimentation test in general medicine. Am. J. M. Sc. 183:643, 1932.

20. Cutler, J. W. : Standardization of sedimentation rate. Am. Rev. Tuberc. 48:314, 1943.

21. Cutler. J. W. : Present concept of erythrocyte sedimentation rate. New Internat. Clin. 3:62, 1940.

22. Cutler, J. W., Park, F. R. and Herr, B. S. : The influence of anemia on blood sedimentation. Am. J. M. Sc. 195:734, 1938.

23. Davis, J. S. : Protein studies in atrophic(rheumatoid) and hypertrophic arthritis.
J. Lab. and Clin. Med. 21:478, 1936.

24. Davis, L. J. : The blood sedimentation rate; a critical clinical survey.
Practitioner 157:13, 1946.

25. Ernstene, C. A. : Erythrocyte sedimentation, plasma fibrinogen and leukocytosis as indices of rheumatic infection.
Am. J. M. Sc. 180:12, 1930.

26. Fahraeus, R. : The suspension stability of the blood.
Acta Med. Scandinav. 55:1, 1921. From Fahraeus 1929.

27. Fahraeus, R. : The suspension stability of the blood.
Physiol. Rev. 9:241, 1929.

28. Feldman, H. : Maintenance of sedimentation rate of erythrocytes in vitro in cases of malignant tumors and Hodgkin's disease.
Am. J. M. Sc. 200:820, 1940.

29. Fischel, K. : The suspension stability rate of erythrocytes in pulmonary tuberculosis and its significance in artificial pneumothorax.
Am. Rev. Tuberc. 10:606, 1925.

30. Gardner, F., Stewart, A. and MacCallum, F. O. : Theraputic effect of induced jaundice in rheumatoid arthritis and allied conditions.
Brit. Med J. 2:677, 1945.

31. Gibson, H. J. : The blood sedimentation rate in rheumatoid arthritis and allied conditions.
Practitioner 157:28, 1946.

32. Gordon, C. M. and Wardley, J. R. : The effect of plasma proteins upon the sedimentation rate of human blood.
Biochem. J. 37:393, 1943.

33. Gorham, L. W. and Thompson, H. C. Jr. : A study of the erythrocyte sedimentation rate and differential leukocyte count in coronary occlusion. New Internat. Clin. 1:144, 1938.

34. Gram, H. C. : Influence of cell volume and percent of hemoglobin on correction of sedimentation reaction. Acta Med. Scandinav. 68:108,1928. From Peters.

35. Greene, R. : Influence of menstruation on suspension stability of red cells. Lancet 2:556, 1941.

36. Greesheimer, E. M., Johnson, O. H. and Ryan, M. : The relationship between sedimentation index and fibrin content in relatively normal individuals. Am. J. M. Sc. 177:816, 1929.

37. Gregg, R. D. : The sedimentation rate in experimental anemia. J. Lab. and Clin. Med. 22:786, 1937.

38. Guliver, G. : On the buffy coat of the blood. Edin. Med. and Surg. J. 64:360, 1845.

39. Ham, T. H. : Critical analysis of methods and factors influencing the sedimentation rate. J. Pediat. 16:681, 1937.

40. Ham, T. H. : Critical analysis of methods and factors influencing sedimentation rate. J. Pediat. 12:678, 1938.

41. Ham, T. H. : Erythrocyte sedimentation; clinical significance and evaluation as a quantitative procedure. J. Clin. Investigation 16:681, 1937.

42. Ham, T. H. and Curtis, F. C. : Sedimentation rate of erythocytes. Medicine 17:447, 1938.

43. Helm, J. D. Jr. : The predictability of the character(increased or normal) of erythrocyte sedimentation rate; a survey of 1000 cases. Am. J. M. Sc. 205:241, 1943.

44. Hertz, J. T. and Rinzler, S. H. : Is the maintained sedimentation rate specific for malignancy?
J. Lab. and Clin. M. 28:323, 1942.

45. Herzog, R. S. : Microsedimentation rate.
J. Lab.and Clin. Med. 27:355, 1941.

46. Hewson, W. : Works ed. by Gulliver
London 1846, pp. 1-90.

47. Hirsh, J. E. : The value of the sedimentation test as a diagnostic aid.
Ann. Int. Med. 10:495, 1936.

48. Homburger, F. : Effect of sodium salicylate on the sedimentation rate of erythrocytes in vitro.
Am. J. Med. Sc. 210:168, 1945.

49. Hubbard, R. S. and Geiger, B. H. : Anemia as a factor in the sedimentation time of erythrocytes.
J. Lab. and Clin. Med. 3:322, 1928.

50. Hunter, J. : A treatise on the blood, inflammation and gun-shot wounds.
Vol. I, Philadelphia: pub. by Bradford
1796, pp. 235-250.

51. Jager, B. V. and Alway, R. : The treatment of acute rheumatic fever with large doses of sodium salicylate.
Am. J. Med. Sc. 211:273, 1946.

52. Jones, T. W. : On the distinction between healthy and buffy blood in minute quantities.
Edinburgh Med. and Surg. J. 60:309, 1843.

53. Kahlmeter, G. : Sedimentation rate of erythrocytes in various rheumatic conditions.
Med. J. and Rec. 128:467, 1928.

54. Kato, K. : Combination micropipette for determination of the sedimentation rate, packed cell volume, fragility of erythrocytes, especially adapted for use in children and small laboratory animals.
J. Lab. and Clin. Med. 23:980, 1938.

55. Kopecky, L. C. : The diagnostic and prognostic value of the sedimentation rate.
Texas State J. Med. 36:302, 1940.

56. Koster, L. : Changes in sedimentation rate in stored citrated blood diagnostic aid in malignant tumors and lymphogranuloma.
Acta Med. Scandinav. 93:420, 1937 From Hertz and Rinzler.

57. Landau, A. : Microsedimentation (Linzenmeier-Raunert method) its serviceability and significance in pediatrics;use of a modified apparatus with simplified technic.
Am J. Dis. Child. 45:191, 1933.

58. Lichty, J. A. Jr. and Hooker, S. P. : Effect of acetyl salicylic acid on sedimentation rate of erythrocytes in rheumatic fever.
Proc. Soc. Exper. Biol. and Med. 48:69, 1941.

59. Lister, J. and Hodgkin, T. : On the structure of the blood.
Phil. Mag. 2:1301, 1827. From McFarlene and O'Brien.

60. Massell, B. F. and Jones, T. K. : Evaluation of the signs of active rheumatic fever with especial reference to the erythrocyte sedimentation rate and leukocyte count.
New England J. Med. 215:1269, 1936.

61. Mathieu, A., Trotman, F. E., Haskins, H. D., Osgood, E. D. and Albert, J. : The sedimentation rate in gynecology and obstretrics.
Am. J. Obst. and Gynec. 21:197, 1931.

62. McFarlene, R. G. and O'Brien, F. R. : The rationale of blood sedimentation rate.
Practitioner 157:1, 1946.

63. McKinley, J. B. and Jackson, R. L. : Comparison of Westergren and Kato erythrocyte sedimentation rate readings.
Am. J. Dis. Child. 67:474,1946.

64. Mendel, D. L. and Korenberg, M. : Maintenance of the sedimentation rate of erythrocytes in cases of cancer, Hodgkin's disease and leukemia.
Canad. M. A. J. 51:353, 1944.

65. Montgomery, L. G. : Detection of a common source of error in the sedimentation test.
Am. J. Clin. Path. Tech. Sec. 7:115, 1943.

66. Morrison, I. R. : Observations on blood sedimentation mechanism.
Am. J. Clin. Path. 11:578,1941.

67. Nichols, D. R. : Clinical significance of erythrocyte sedimentation rate.
M. Clin. North America 29:936, 1945.

68. Obermer, E. : Clinical significance of erythrocyte sedimentation rate with a plea for adaption of a simple technique.
Practitioner 151:43, 1943.

69. Payne, W. W. : Acute rheumatism and the sedimentation rate.
Lancet 1:74, 1932.

70. Peters, J. T. : Determination of sedimentation rate of red blood cells.
Arch. Int. Med. 75:105, 1945.

71. Ponder, E. : Blood sedimentation.
Medical Physics, 1408, 1944.

72. Rabinowitz, M. A., Shookhoff, C. and Douglas, A. H.: The red cell sedimentation time in coronary occlusion.
Am. Heart J. 7:521, 1931.

73. Risemman, J. E. F. and Brown, M. G. : The sedimentation rate in angina pectoris and coronary thrombosis.
Am. J. Med. Sc. 194:392, 1937.

74. Rogatz, J. L. : A simple micrometric apparatus for determing the sedimentation of erythrocytes.
J. Lab. and Clin. Med. 28:1842, 1944.

75. Rourke, M. D. and Ernstene, A. C. : A method for correction the erythrocyte sedimentation rate for variation in the cell volume percentage of blood.
J. Clin. Investigation 8:545, 1930.

76. Rourke, M. D. and Plass, E. D. : An investigation of various factors which affect the sedimentation rate of the red blood cells.
J. Clin. Investigation 7:365, 1929.

77. Rubin, E. H. and Smith, N. N. : Relation of the hemoglobin, cell count and cell volume to the erythrocyte sedimentation reaction.
Arch. Int. Med. 39:303, 1927.

78. Sappington, S. W. and Gillis, L. M. : Heparin as the anticoagulant in sedimentation tests.
Am. J. Clin. Path. 11:83, 1941.

79. Schlesinger, O. : The blood sedimentation rate in rheumatic fever.
Practitioner 157:38, 1946.

80. Sellards, A. W. : The effect of heated serum on rouleaux formation of red blood corpuscles.
Bull. John Hopkin's Hosp. 19:271, 1908.

81. Shookhoff, C., Douglas, A. H. and Rabinowitz, M. A.: Sedimentation time in acute cardiac infarction.
Ann. Int. M. 9:1101, 1936.

82. Smith, C. H. : A method for determining the sedimentation rate and red cell volume in infants and children with the use of capillary blood.
Am. J. M. Sc. 192:73, 1936.

83. Stokes, J. B. : The diagnostic and prognostic value of erythrocyte sedimentation rate in general proctice.
Ill. Med. J. 75:236, 1939.

84. Struthers, R. R. and Bacal, H. L. : Determinations of the activity of rheumatic fever infection in childhood.
Canad. M. A. J. 29:470, 1933.

85. Todd, G. S. : The blood sedimentation rate in pulmonary tuberculosis.
Practitioner 157:36, 1946.

86. Vogt, C. T. : The erythrocyte sedimentation reaction during pregnancy.
Am. J. Obst. and Gynec. 41:206, 1941.

87. Walton, A. C. R. : The corrected erythrocyte sedimentation test.
J. Lab. and Clin. Med. 18:711, 1933.

88. Walton, A. C. R. : The erythrocyte sedimentation test; a clinical and experimental study. Quart. J. Med. 26:79, 1933.

89. Wasson, U. P., Brown, E. E. and Weintraub, C. : The blood picture in rheumatic fever. Am. Heart J. 221:342, 1941.

90. Weiss, A. : A prognostic value of the sedimentation rate in arthritis; a modification of the technique. Am. J. M. Sc. 181:379, 1931.

91. Weisz, A. and Taran, L. M. : The finger puncture method for sedimentation rate. J. Pediat. 22:565, 1943.

92. Westergren, A. : The technique of red cell sedimentation reaction. Am. Rev. Tuberc. 14:94, 1926.

93. Wharton, J. T. : Observations on some points in anatomy, physiology and pathology of the blood. Brit. and Foreign Med. Rev. 14:585, 1842.

94. Whitley, L. E. H. and Hynes, H. : Correction of the sedimentation rate in anaemia. Lancet 2:249, 1938.

95. Wintrobe, M. M. : The erythrocyte sedimentation test. Internat. Clin. Series 46, 2:34, 1936.

96. Wintrobe, M. M. : The erythrocyte sedimentation test. Am. J. Clin. Path. 11:562, 1941.

97. Wintrobe, M. M. and Landsberg, W. J. : A standardized technique for the blood sedimentation test. Am. J. M. Sc. 189:102, 1935.

98. Wood, P. : The erythrocyte sedimentation rate in diseases of the heart. Quart. J. M. 5:1, 1936.

99. Yardumian, K. : Physicochemical factors influencing the red cell sedimentation rate. Am. J. Clin. Path. 7:105, 1937.