United States Patent [19]

Bull

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[45] **July 23, 1974**

[54] **METHOD FOR SEDIMENTATION STUDY**

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[21] Appl. No.: **191,886**

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 113,166, Feb. 8, 1971, abandoned.

[52] **U.S. Cl.** ..... **73/61.4,** 23/230 B, 73/64.1

[51] **Int. Cl.** **G01n 15/04,** G01n 33/16

[58] **Field of Search** ..... 73/61.4, 64.1, 61 R; 233/26; 23/230 B

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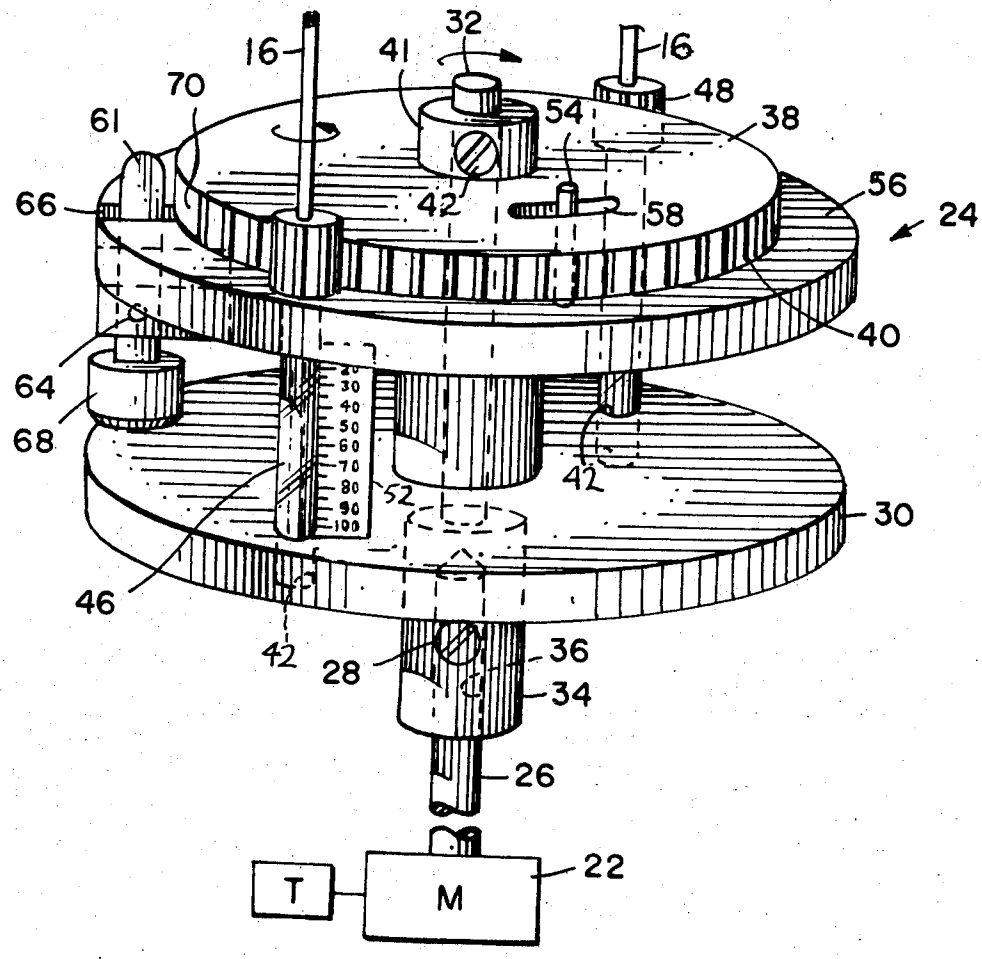
[57] **ABSTRACT**

Method for the study of the sedimentation characteristics of whole blood comprising the steps of cyclically applying greater than gravity force laterally to a thin, substantially vertically oriented column of whole blood, and rotating the column about 180 degrees about its own axis between each cycle. The sample columns are placed in tubes arranged with their long axes oriented substantially parallel to the axis of rotation of a centrifuge and the tubes are rotated about their own long axes between each cycle and only when at or substantially at rest. A preferred test operation using four cycles of 45 second duration is described with the rotation of the columns being effected by reversal of the direction of rotation of the centrifuge head at the end of each cycle. Other cycle durations are described for providing results correlatable with standarized sedimentation test procedures.

25 Claims, 12 Drawing Figures



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SHEET 1 OF 4

FIG. 1

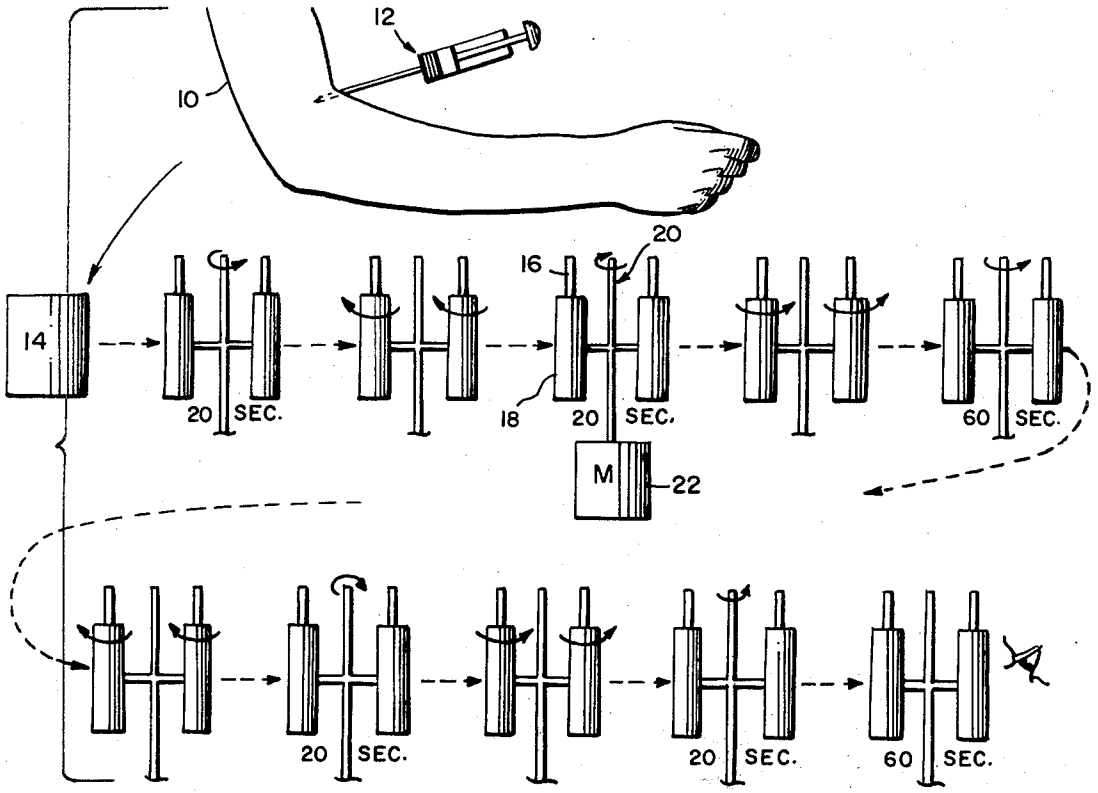

FIG. 2

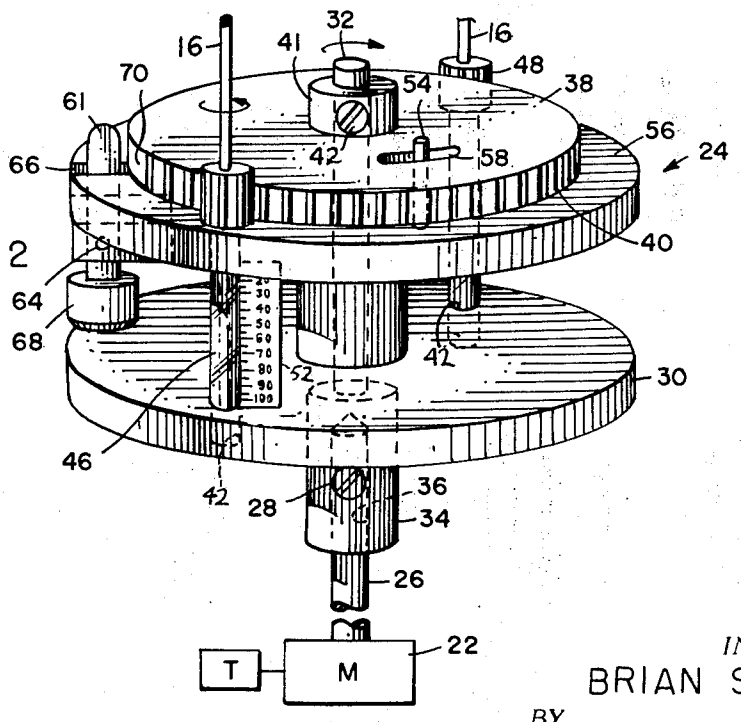

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FIG. 3

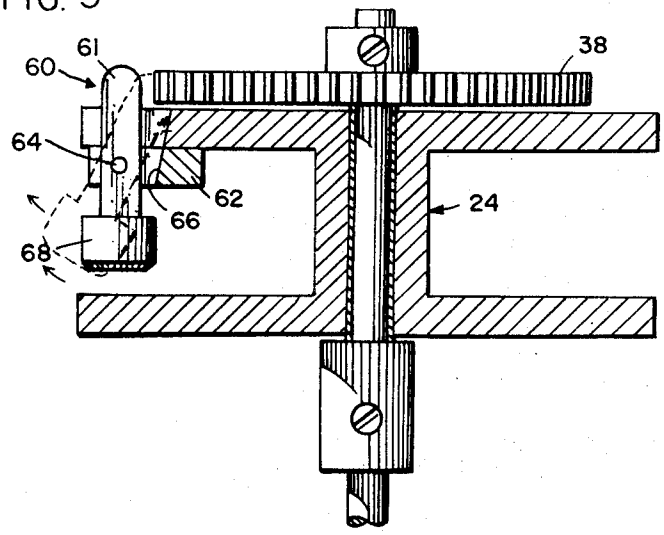

FIG. 4

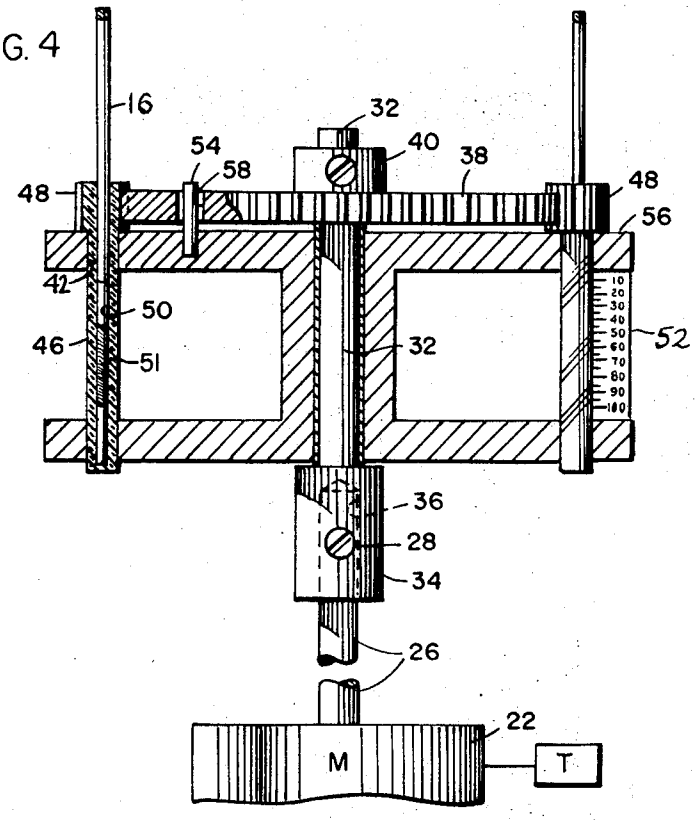

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FIG. 5

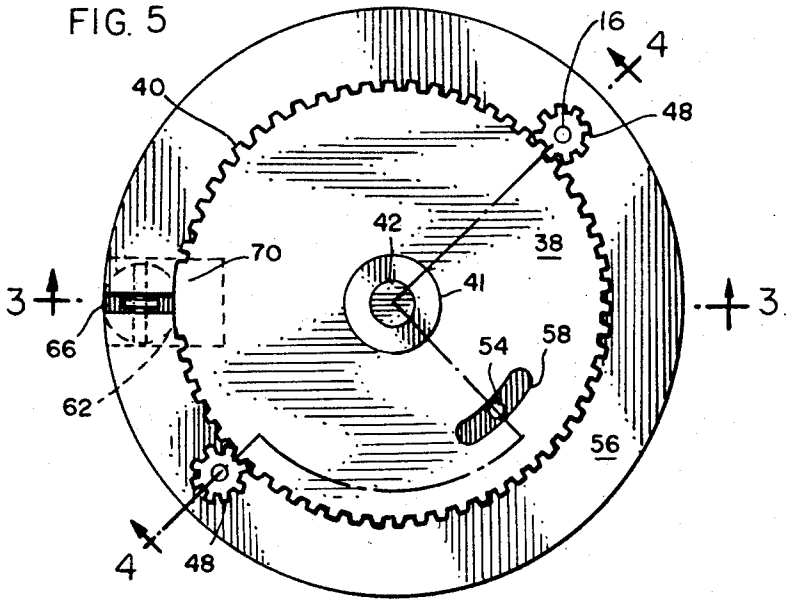

FIG. 6

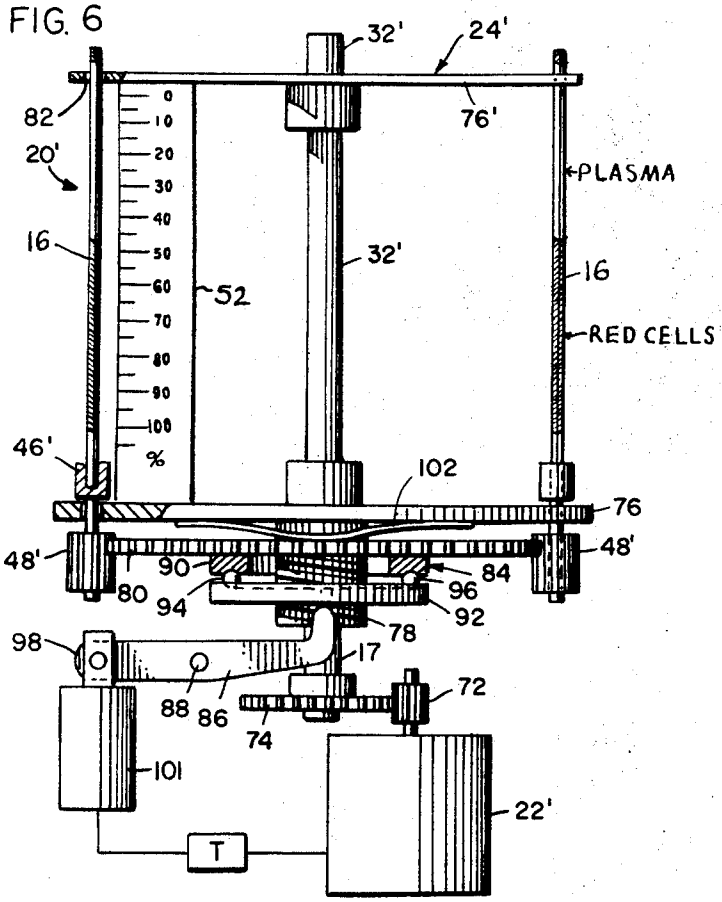

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FIG. 7

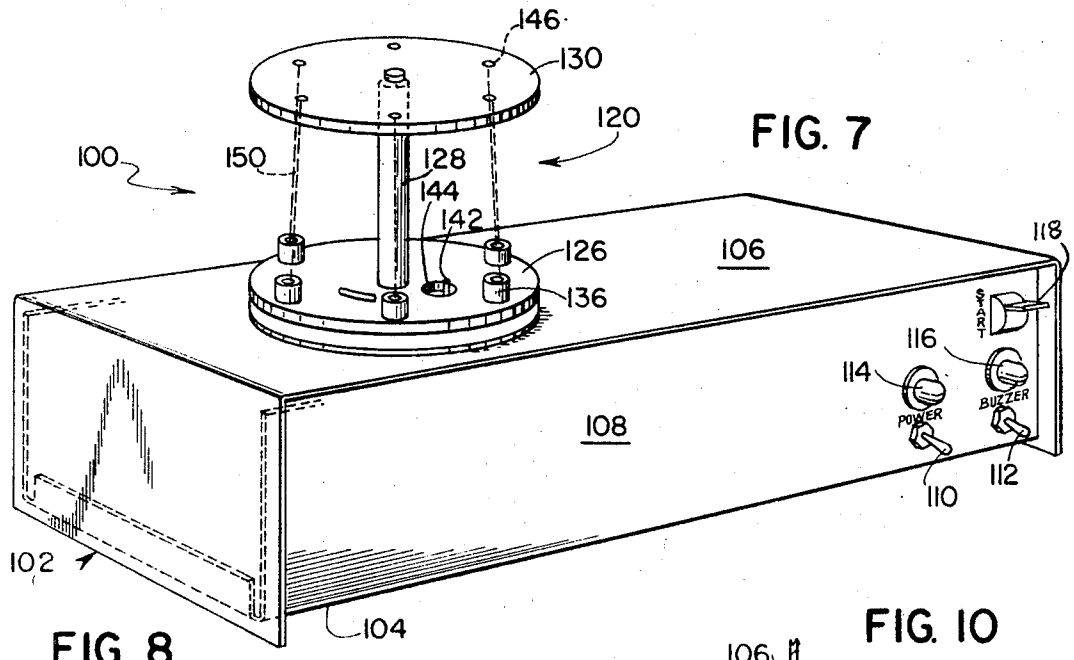


FIG. 8

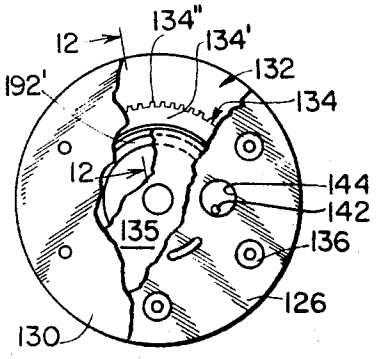


FIG. 9

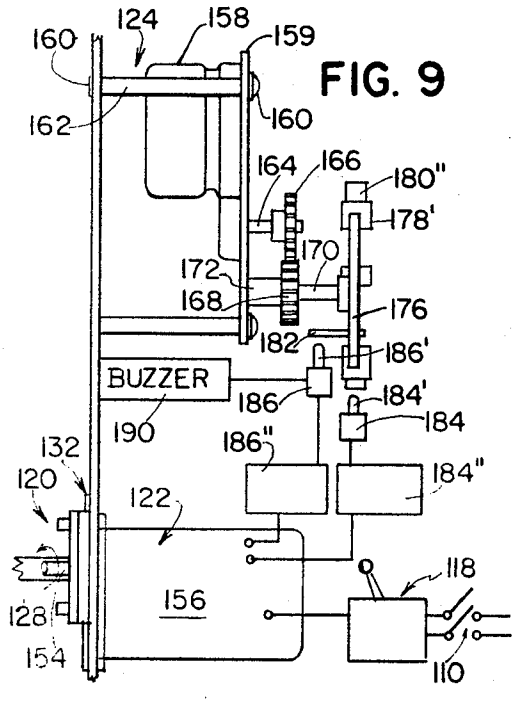


FIG. 10

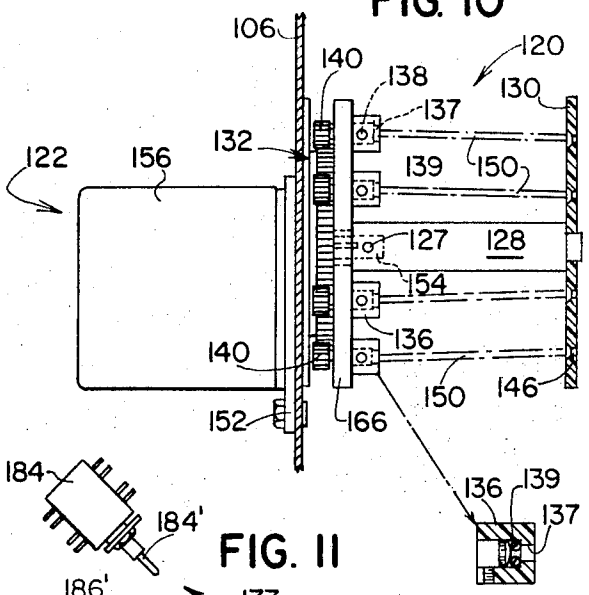


FIG. 11

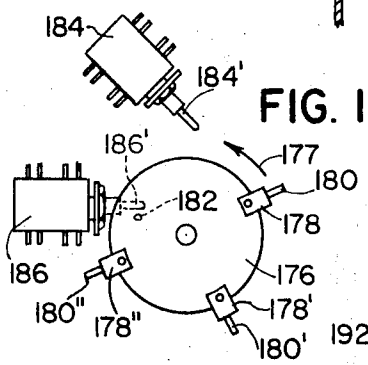
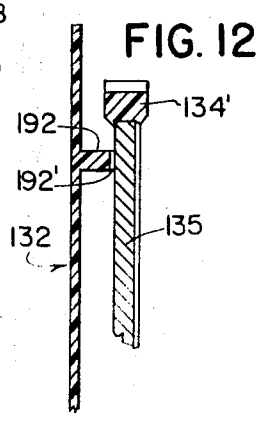


FIG. 12



METHOD FOR SEDIMENTATION STUDY

CROSS-REFERENCE TO RELATED APPLICATION

This application is a continuation-in-part of my copending application, Ser. No. 113,166, filed Feb. 8, 1971, entitled "SEDIMENTATION RATE TEST METHOD AND SEDIMENTATION RATE CENTRIFUGE THEREFOR" now abandoned.

FIELD OF THE INVENTION

This invention relates generally to diagnostic examination of whole blood and more particularly concerns the provision of improved and means for whole blood sedimentation study.

BACKGROUND OF THE INVENTION

It is well known that the suspension stability of whole human blood is altered in the presence of many functional disorders. The determination of this characteristic generally has been effected by performance of well-known standardized sedimentation tests in the course of clinical analysis. Using the sedimentation test results, the presence of more or less occult disease can be brought to medical attention. Such results particularly are of importance in the differential diagnosis as between functional disorders having closely similar symptomatic manifestations, as well as in supplying a guide to the progress of certain diseases. Accordingly, it is believed that substantial benefit could be obtained in the diagnosis and treatment of medical disorders by the establishment of sedimentation study procedures which would produce comparative information quickly and economically so that a sedimentation study could become a routine procedure in clinical examination. However, as practiced presently, the sedimentation test is too time consuming, too affected by laboratory introduced artifacts and subject to misinterpretation in anemic individuals, so that the test is not a test offered to every patient as a routine clinical test procedure such as a blood count, for example.

Present methodology involves essentially the mixing of a whole blood sample with a selected anticoagulant, introducing this well mixed sample in a vertically arranged glass tube and permitting the red cells of the sample to sediment under the influence of gravity. This process is slow, usually taking sixty or more minutes. The only accepted variations in this method takes the form, singly or in combination, merely of changing the length of the glass tubes employed, varying the bore of such tubes, careful selection of the anticoagulant employed and/or modification of the degree of dilution utilized. None of these variations have alleviated the principal drawback to adoption of the sedimentation test as a routine procedure, this drawback being that present sedimentation rate tests methods are too time consuming for routine employment or mass studies.

Another important deterrent to adoption of sedimentation testing as a routine procedure has been the extreme sensitivity of this test to the arrangement of the test sample in an absolutely vertical orientation for the duration of the test. It has been found that a sample column which is oriented at only a three degree offset from vertical will result in inconsistent acceleration of the sedimentation rate and reduces the relative differences between the comparative normal and abnormal

blood sedimentation characteristics, thereby reducing the value of the test in diagnosis.

Accordingly, it is the principal object of the invention to provide an improved sedimentation study method for whole blood which meets the requirements for rapidity, economy, accuracy and reliability essential for adoption as a mass applied clinical laboratory test procedure, and, concomitant therewith, to provide a sedimentation rate centrifuge particularly adapted for implementing and carrying out the steps of the improved study method.

Another object of this invention is to provide an improved sedimentation study method which provides comparative information on the sedimentation behavior of whole blood from normal persons and from persons suffering from functional disorders, this information being provided quickly and with reliability, the method being free from the sensitivity of prior methods to dispostion of the test samples during performance thereof; and also which results can be obtained approximately equivalent to standard methods of sedimentation testing and also can provide a sedimentation study result independent of hematocrit effect of the sample.

SUMMARY OF THE INVENTION

A sedimentation study method for whole blood comprising the steps of applying greater than gravity force less than 8.25 G laterally to a substantially vertically oriented column of whole blood sample in a repeated series of applications and rotating the column about its own vertical axis between each application of force; thereafter determining the concentration of cells in the resulting packed portion of said sample. According to the subject method, a comparison is made between the start level and the treated level. The column may be then fully packed by centrifugation at 100 G or the like and a comparison again made to the treated column level. A ratio then is determined of the two results to provide a hematocrit independent value. A centrifuge apparatus is provided for implementing the subject method, comprising a centrifuge head and a motor, the centrifuge head carrying at least a pair of sample tube holders arranged to orient the samples in substantially vertical columnar array, drive means connected between the motor and the centrifuge head for rotating same, means associated with the head and tube holders for rotating the tube holders about their own vertical axes and timing means operable on said drive means for operating the centrifuge head in timed cycles with the centrifuge head being brought substantially to a rest condition between cycles and means to restrict the rotation of the tube holders about their own vertical axes to periods during which the centrifuge head is at substantial rest condition.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a diagrammatic representation illustrating an improved sedimentation rate study method according to the invention;

FIG. 2 is a perspective view of the sedimentation rate centrifuge constructed in accordance with the invention;

FIG. 3 is a vertical section taken through the centrifuge of FIG. 2 along the lines 3—3 and in the direction indicated;

FIG. 4 is a vertical section taken through line 4—4 of FIG. 2 and in the direction indicated;

FIG. 5 is a top plan view of the centrifuge arrangement as shown in FIG. 2;

FIG. 6 is a vertical section taken through a modified embodiment of the invention;

FIG. 7 is a perspective view of a further modified embodiment of the invention;

FIG. 8 is a top plan view of the centrifuge arrangement illustrated in FIG. 7 with portions broken away to show interior detail;

FIG. 9 is a diagrammatic representation of the embodiment illustrated in FIG. 7;

FIG. 10 is a bottom view of the timing means utilized in the embodiment of FIG. 7;

FIG. 11 is an elevational view of the centrifuge head of the embodiment illustrated in FIG. 7;

FIG. 12 is a fragmentary sectional view taken along line 12—12 of FIG. 8.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The method of studying the sedimentation characteristics of whole blood in accordance with the invention, capitalizes in part upon the fact that the blood from a normal, healthy individual has greater suspension stability than does blood from a sick individual. Three phases are known to occur during the sedimentation of whole blood. The first is characterized as the phase of rouleaux formation. During this phase, the red cells of the whole blood stack together in what is defined in the art as rouleaux. This phase occupies the first few minutes subsequent to filling of the sedimentation tube with sample. Next begins the phase of maximum sedimentation wherein after about three to five minutes, the red cell rouleaux reach their maximum velocity of fall. This velocity is dictated by the average density of the rouleaux and the viscosity of the plasma through which they are falling. The last phase concerned is characterized as the packing phase. As the rouleaux reach the bottom of the sedimentation tube, they pack and, as a consequence, the average rate of fall decreases and eventually, when packing is complete, no further change occurs.

The samples from both normal and sick individuals, given enough time, will pack to approximately the same extent; but the blood samples from the ill patient goes through the rouleaux formation phase and into the phase of maximum velocity of fall more rapidly than does the blood from a healthy individual. An isolated red cell is so small a particle that even though its density is considerably greater than that of the plasma, the large relative surface area becomes the over-riding factor and an isolated red cell is plasma falls very slowly under the influence of gravity. The rate of fall of red cells is thus governed almost entirely by the size of the rouleaux which they form. The blood from a healthy individual forms such rouleaux much more slowly than does blood from a sick person. If both samples are set up simultaneously, there is a period of time when application of force greater than gravity to the blood will affect the blood from a healthy individual minimally and that from a diseased patient maximally. The crucial or critical time is obvious when the healthy blood has just only begun to form rouleaux and the sick blood has formed large rouleaux which have already begun to sediment.

It had been critical to the study of sedimentation rate that the conventional test must be performed under conditions where the sample column is disposed in absolutely vertical orientation. A tilt as little as three degrees from vertical under gravity will accelerate the sedimentation rate considerably, and decrease the relative differences between normal and abnormal blood. It is believed that this effect is due to the fact that red cells falling through the plasma hit the walls of the container and roll down the walls permitting the plasma free egress from the depth of the sample. Whenever the plasma is forced to traverse the descending column of red cells, the sedimentation rate is slowed. Normal red cell rouleaux, fall much more slowly than do abnormal red cell rouleaux, probably because the forces holding them together are weaker and the upsweeping plasma either breaks them up or prevents them from forming large enough clumps to sediment rapidly. Accordingly, by applying a greater than gravity force to the sedimenting blood, according to the invention, the ascending plasma is forced to traverse the descending red cells. As will be explained, a slight inclination of the column up to about 6° from vertical is permissible with the method of the invention, particularly to avoid spilling of the sample during the run.

According to the invention, greater than gravitational force is applied laterally to a long thin column of whole blood sample by rotation thereof in a centrifuge capable of delivering a force in the range of 2 to 12 G with the test taking from one-half hour at a minimum G to about one minute at the high end of the aforesaid range. The higher the G Force, the shorter the elapsed time of the test. The net effect is to force the red cells to traverse the plasma component of the blood over a very short distance since the effective cross-sectional area of the tube is now vast relative to the wall surface area and the red cells cannot collect in one portion of the tube against the wall so as to permit the plasma to escape freely elsewhere. Insofar as the sedimentation process is concerned, the long thin column has been transformed to a shallow wide diameter "pool" and since the force is directed substantially perpendicular to the long axis of the column, the problems of channeling heretofore experienced in sedimentation testing are obviated.

The laterally applied force acts to pack the red cells rapidly, permitting the plasma physically to change location therewith and approach the final packing state over a much shorter time period, than normally would be expected under gravitational force.

One example utilizing the method according to the invention, permits the completion of the packing stage under gravity subsequent to the periodic application of the greater than gravity force and the level of the packed red cell rouleaux observed and compared with that of a normal blood standard sample treated under the same conditions.

Another example of the subject method involves the obtaining of a packing factor after the said periodic cycles and a second packing factor on maximum packing by centrifugation under more than 100 G. The ratio of these factors is a value indicative of the presence of asymmetric protein, a fact important to state of health evaluations and independent of hematocrit.

The selection of the duration of the centrifugation cycles as well as their number are dependent also upon the speed of the drive or synchronous spinner motor

utilized, the diameter of the column and of the sedimentation tube utilized, and the degree of cant or tilt permitted.

Another method of practicing the invention involves a program selected to use four cycles of applied force laterally to the column and of 45 second duration each. Between the first and second, the second and third and the third and fourth cycle of centrifugation, the columns are rotated about their own vertical axes. Care is taken to assure that the axial rotation of the columns take place only when the force applied laterally to the long axes of the columns is less than one G. This condition occurs when the column is at rest, substantially at rest or, to put it another way, begins its translation in its circumferential rotation with and on the centrifuge head. A friction or similar drag is applied directly to the head, that is, to the spool on which the columns ride, at the time each cycle is ending so that a gradual braking is effected so that the centrifuge head may be stopped without abrupt rotation of the columns. Abrupt stoppage of the columns must be avoided, and likewise, jarring or other sharp disturbance of the columns are avoided so that the cells which have separated from the plasma and gathered at the inner wall of the tube containing the test sample column will not be broken away from each other or from the tube wall. The cells in the column must rotate with the tube, and the column as the same is rotated in accord with the invention.

In accordance further with the method of the invention, between each application of greater than gravity force, the test sample column is rotated about its own long axis, preferably 180°.

This seems to provide beneficial results in obtaining reproducible packing in alternatively permitting resuspending the cells by rotating the tube and its column contents and again effectively forcing those cells back through the plasma. Thus, according to the invention, cohesive forces are utilized during the centrifugation under the relatively low G force to force the cells against the wall and dispersion forces are utilized when the cells are forced back through the plasma by again centrifuging but after 180° rotation of the tube and column of test sample.

Clearly, the rotation of the tube containing the test sample column on its axis must be sufficiently gradual so as not to sever the adhesion between the column of cells and the tube wall. Sharp rotation will leave the cells, while the wall moves. Since the purpose generally of the aforesaid column rotation is to effectively force the cells through the plasma component of the sample so as to provide a reasonably reproducible packing factor, movement of the cells with tube wall during said tube rotation on its axis is mandatory.

Now referring to the diagrammatic flow chart of FIG. **1**, attention is directed first to the sedimentation test method according to the invention. Following this method will provide test results equivalent to the Wintrobe standard method of "sed.rate testing." The blood sample S is taken from the patient **10** by means of a syringe **12** or the like, transferred to a vessel and mixed with any one of a plurality of selected anticoagulants. A predetermined amount of mixed sample is placed in a long, thin tube, sometimes referred to in the art as a microhematocrit tube **16** and which constitutes the test sample. The tube **16** is filled to a standardized "depth." The steps involved in the production of this test sample are well known in the art and is represented by box **14** in FIG. **1.** The microhematocrit tube **16** generally of 2 mm diameter is placed substantially vertically in one of the tube holders **46** of the centrifuge **20.** The other holders are likewise employed with other samples so that the centrifuge is balanced. As will be discussed later, other configurations of tube holders are contemplated.

The range of speed of rotation of the centrifuge **20** preferably is selected between 200 and 1,000 revolutions per minute so that an effective centrifugal force of approximately 7.25 is provided. The motor **22** of the centrifuge **20** is energized and the centrifuge is caused to operate, here in a clockwise direction, for a relatively short spin, say about 20 seconds. The centrifuge then is stopped and brought to rest. When the centrifuge is at rest or at least substantially at rest, the tube holder **18** and its tube **16** are caused to rotate about its own long axis 180° and the centrifuge **20** again is caused to rotate, applying a force of approximately 6.25 to 8 G to the vertically oriented column of test sample in tube **16.** This second rotation also is for a short duration, again about 20 seconds, after which the centrifuge **20** is brought to a rest condition and the tube holder **18** and the tube **16** therein again are rotated 180° about its own axis. A reversing motor is used so that the centrifuge's direction of rotation is changed with each cycle. The rotation of the test column about its own axis may be in a direction opposite to the immediately preceding direction of rotation of the centrifuge **20** because it is easier to effect rotation of the tube and tube holder about their own long axes due to the inertia of the centrifuge head in starting. A unidirectional centrifuge also can be used with the direction of rotation of the tubes and tube holders about their own axes remaining unchanged from cycle to cycle.

The first two short spins are for the purpose of accelerating rouleaux formation by shunting the red cells back and forth through the plasma to cause them to collide without really effecting the sedimentation thereof. During these spins, the red cells are either isolated or at most in groups of two to four cells and as a result they are not moved any appreciable distance by the approximately 6.25 to 8 G force applied perpendicular to the long axis of the tubes during the first two short spins. The cells are moved further than they would move under gravity influence during this period and collisions between individual cells for the formation of rouleaux accelerated. After the pair of short spins, the rouleaux formation in blood from an ill person has taken place to a larger extent than it would if the blood sample originated from a normal or healthy person.

After the two short spins of about 20 seconds each and the rotation of the test column about its own vertical axis each thereafter, the rouleaux formation is such that the approximately 6.25 to 8 G force may be applied when the test sample is experiencing the second phase of sedimentation, that is the maximum velocity of descent of the formed rouleaux in the test sample from the ill person, a time whereat the sedimentation rate can be most effectively accelerated and compared to the reaction of the standard or normal test sample wherein rouleaux formation is barely initiated.

The centrifuge **20** is caused to rotate again to apply a force of approximately 6.25 to 8 G perpendicular to the long or vertical axis of the upstanding sample col-

umn, but this time, the application of said G force laterally to the column is continued for a period of about 60 seconds, a long enough period of time to move the red cell rouleaux physically to one side of the tube. After this longer centrifugation, the tubes and tube holders once again are brought to rest condition, and the said tubes and tube holders again are rotated 180° about their own axes. Two more 20 second spins follow with intermediate rotation of the tubes and tube holders about their own vertical axes. These final two spins are intended to aid the red cell column to re-establish itself in its vertical tube so that its level can be read. The rouleaux layer is permitted to fall free of plasma hindrance. The plasma component is permitted to escape from the red cell rouleaux column held against the tube wall. No hindrance to such passage can be expected since the cell column is held against the wall of the tube by the approximately 6.25 to 8 G force while the 1 G natural gravitational force applied axially causes them to travel to the bottom of the tube. The tubes, having been filled to a standard depth, the level of the packed cells is read by comparison to a fixed scale either mounted on the centrifuge head as shown in FIGS. **2, 6** and **8** for example, thereby being compared to the level of the packed cells observed in a standard or normal blood sample which had been treated similarly or simply matched to a coded chart carried on a separate card.

Following the method of the invention, at a force of about 6.25 to 8 G, the test duration after which meaningful test results can be observed is about 3 minutes as comapred to convention sedimentation methods where meaningful results can only be reached after 60 minutes.

It should be understood that the higher than G force applied, the shorter the elapsed time for the test. For example, the following time G table is appropriate:

TIME (Minutes)	APPROXIMATE FORCE in G's
30	2–3
15	5
4	7
2	9
1	10–12

Preferably the range of G force applied in the course of operating the particular embodiment is approximately 6.25 to 8 G's with a generally preferred force application of 7.25 G.

One of the substantive disadvantages of conventional sedimentation test methods is the criticability of vertical orientation of the test column. With the method of the invention the slight inclination of the column is permissible. Inclination of the tubes from the vertical such that the lower end of the tube is further from the axis of rotation of the centrifuge head introduces a component of force up the tube and therefore in opposition to the gravitational force which is tending to move the particles down the tube. It thus slows the sedimentation process and increases the amount of time required to perform the test. Provided the inclination of the tube from the vertical is not excessive, there is no effect upon the test other than the increased time required ordinarily. Excessive can best be defined in the following manner. If the rotation of the centrifuge head is subjecting the tubes to a horizontal acceleration of 8 G, there will be a component of this force up the tube of 8 G times tangent theta where theta is the inclination between the tubes and the vertical. In this example, as the angle theta approaches 6°, the upward component approaches 1 G. At 1 G there is effective neutralization of the gravitational force as long as the centrifuge is spinning and during this time period, which occupies slightly more than half of the usual cycle, the cells will move neither up nor down the tube; they will of course move outwards. For remainder of the cycle the cells are under 1 G and will, of course, travel downwards; the net effect is simply to prolong the time required to perform the test. The time gets shorter and shorter as the tubes are returned more and more to the vertical position and indeed continues to shorten if the tubes are inclined with their top ends inwards. But with further inclination beyond about 6° instability and irreproducibility become a problem. The effective limits therefore are from vertical with an inward or outward cant of approximately 6° if the force applied is in the range of 8 G's. Thus one can state that the greater than gravity force (relative centrifugal force) is applied to a column inclined at an angle theta from the vertical such that the relative centrifugal force applied times the tangent of the angle theta is a value equal to or less than 1.

The cycles set forth as an example in FIG. **1** give results in terms of a sedimentation rate correlative with and equivalent to the value obtained by following the well known Wintrobe method of sedimentation rate determination. A result approximately equivalent to other standard method values can be obtained by variation of the number and duration of the cycles.

One problem which is encountered in interpreting sedimentation rate inforamtion as applied to medical diagnosis is the effect that the hematocrit has upon the sedimentation rate and the difficulty in ascribing the effect of an anaemic condition upon the observed value. It is extremely difficult to apply corrections to observed sedimentation rates to correct for the effect of the hematocrit thereupon. An anaemic blood sample may be observed to have a certain sedimentation rate which otherwise may indicate an abnormal functional condition or which may be due to the anaemia condition. Correction for the effect of hematocrit upon the sedimentation rate observed was not possible routinely using conventional sedimentation rate testing methods. However, it has been observed that the effect of hematocrit in the method according to the invention is a linear one and a hematocrit correction chart can be constructed. A simple mathematical correction to all observed sedimentation rates observed following the method of the invention whereby all sedimentation rates can be reported at a standard or normal hematocrit, say for example, 45 percent. Thus heretofore experienced misinterpretation of the results in anaemic individuals may be eliminated.

In lieu of the standardized sedimentation rate values obtained, say pursuant to the method outlined in FIG. **1**, one following another example of such method may obtain information independent of the hematocrit factor. Here, the cycles are of substantially of equal duration; in one example, 45 seconds under the 6.25 - 8 G applied in four cycles with rotation of the tubes and columns about their own vertical axes between each cycle. A ratio of initial to packing level is taken. The tubes may then be packed to a maximum extent by application of G force of at least 100 G and a ratio of the first and maximum packing levels taken. The ratio of

the resultant maximum packing factor and the first packing factor is taken with the resultant value, here termed a ZSR, a value independent of hematocrit fluctuations.

In summary, in following the method of the invention as described above, the red cell sedimentation has been accelerated by increasing the greater than gravity force applied at the time when abnormal blood would be most sensitive to the multi G effect than blood from a normal healthy person, this time being during the maximum velocity phase of sedimentation reached prior to the time it would be reached if the sample were from a normal healthy person. Further, the multi G force is applied laterally to a thin vertically oriented column of blood sample to obviate the problems of channelizing occurring where sedimentation does not act absolutely parallel with the walls of the vessel containing the sample column; the container effectively being transformed from the long thin vertically arranged tube to one which has a wide diameter. The intermittent rotation of the column about its own vertical axis between the centrifugal spins is intended to hold the red cells on one side of the column while permitting the plasma to move on the other side so that, in effect, the red cells are repeatedly forced to move through the plasma with a reproduced packing factor being the ultimate result.

The performance of the method above described required a centrifuge capable of applying the preferably G force in the range of 6.25 to 8 G in separate cycles of predetermined duration. The centrifuge is required to rotate the tube containing the sample in a circumferential path about the axis of rotation with the tube being in substantially vertically oriented disposition parallel to the axis of rotation of the centrifuge head although a cant from vertical of up to 6° is permitted, contrary to standard sedimentation rate methods. Additionally, the centrifuge is required not only to permit rotation of the tube along said circumferential path for a predetermined length of time and then the tube periodically must be brought at least to a momentary substantially rest or stationary condition, then rotated about its own axis a predetermined number of degrees, and the rotation of the tube along said circumferential path resumed for the next cycle of greater than G force application. At least, the rotation of the tube about its axis must not occur during the application of greater than G force.

The centrifuge also should have timing means T for selectively controlling and/or varying the duration of the cycles. According to the method of FIG. **1**, the duration of the successive cycles are not equal but follow a definite program. Another example of the subject method has successive equal duration cycles. The greater the G force, the shorter the cycles and total elapsed time. In addition to the means required to rotate the individual test sample holders between cycles when the centrifuge is brought to a substantially rest condition, the centrifuge must be provided with means whereby the tube holder is brought to rest or at least substantially to rest in its travel along its circumferential path before the rotation of the tube holder about its vertical axis can take place. This feature is required so that the contents of the sample tube carried by the tube holder, that is, the column itself, will rotate with the rotation of tube and tube holder. Too rapid angular acceleration or rotation centrifugally tends to result in slippage, the contents of the sample tube having a tendency to remain stationary while the tube and tube holder rotate. This must be avoided. According to the method of the invention, the column contents of the sample tube must rotate axially with the tube wall and care must be taken to assure only such rotation.

The centrifuge **20** constructed in accordance with the invention and illustrated in FIGS. **1** to **5** comprises a motor **22,** generally one which is reversible, for rotating a head **24** in the range of 200 to 1,000 R.P.M. Generally, the speed of rotation can be varied easily by selection of heads of different diameter. The effective force output preferred is in the range of 6.25 to 8 G. The shaft of the motor **22** is coupled to the head **24** by fastening means **28.**

The head **24** comprises a spool **30** mounted for rotation on a shaft **32.** The shaft **32** has an enlarged end **34** having a passage **36** to receive the shaft **26** of the motor **22.** A spur wheel **38** having circumferential teeth **40** is mounted at the opposite end of shaft **32** coaxial with the spool **30.** A locking ring **41** is fastened to the shaft **32** by fastening means, such as screw **42.** The spur wheel **38** rotates with the shaft **32.** The spool **30** carries a plurality of openings **44** circumferentially disposed to receive tube holders **46.** The tube holders **46** are arranged in diametrically opposed pairs for balance, only one pair being shown in the FIGS. **1–6** for convenience. Each of the tube holders **46** is provided with a pinion wheel **48** either secured frictionally or otherwise thereto or integral therewith. The body of holder **46** may be transparent so that the tube **16** may be viewed and a graduated scale **52** mounted on the spool **30** adjacent the tube holder for reference in reading. The openings **44** are so arranged that the pinion wheels **48** mesh with the circumferential teeth **40** of the spur wheel **38.** Each tube holder **46** is constructed with a top opening bore **50** capable of receiving in vertical orientation, the microhematocrit tube **16** which contains the sample of blood to be tested. Each tube holder **46** may have a leaf spring **51** within the bore as an aid in maintaining the proper disposition of the tube. An upstanding pin **54** is secured to the upper disc **56** of the spool and a slot **58** is provided in the spur wheel **38.** Slot **58** is configured in the form of a segment of an arc, as shown in FIG. **5.** The length of the slot is selected to assure that the rotation of the pinion wheel **48** is taken through exactly 180°. The spool **30** is mounted to the shaft **32** so that it is freely rotatable, the spur wheel **38** being secured so that it rotates with shaft **32.** After the pin **54** reaches the end of slot **58,** no further rotation of the pinion wheel **48** can take place. The motor **22** is brought to rest and then started in the reverse direction. At this time, the pinion wheel **48** must first rotate, owing to the inertia of the spool **30,** until the pin **54** reaches the other end of the slot **58.** Thus, the carrier tube and hence the microhematocrit tube **16** is caused to rotate exactly 180° with each reversal of the driving motor **22.**

Deceleration of the motor **22** before it stops, has the same effect on the spool **30** as the reversal of the motor, so that the 180° rotation of the tube **16** takes place before the tube **16** comes to rest. The tubes must be at rest before rotation about their own axes to avoid rotation only of the tubes rather than the column. This problem can be overcome by introducing a constant drag on the spool **30** such as, for example, a magnetic induction brake or a friction pad (not shown).

A preferred method of alleviating the said deceleration effect is the provision of an interlocking device designated generally by reference character **60** (FIG. 4). The interlocking device **60** comprises a metal strip **61** pivotally secured to a block **62** which, in turn, is attached to the inside wall of disc **56** of the spool **30.** The strip **61** is pivoted as at **64** to move in radial slot **66.** A weight or mass **68** is provided at the lower end of strip **61** and secured thereto. When the spool **30** is rotating, the mass **68** is held radially outward of the spool **30** so that the strip **61** engages the spur wheel **38.** This condition remains until the motor speed falls to zero. At this time, the mass **68** falls to its rest position and the strip **61** disengages from the wheel **38.** It should be noted that the teeth of the spur wheel between the correct engagement positions are omitted as indicated at **70** in FIG. **5.** In lieu of the pin and slot arrangement specifically illustrated in FIGS. **2, 4** and **5,** one can utilize a spoked wheel gear instead of wheel **38,** with a pair of pins disposed between the spokes so as to limit the rotation of the pinion gear **48** and with it, the sample tube and test column therewithin.

In FIG. **6,** there is illustrated a sedimentation rate centrifuge **20** which has been modified so as to obviate the need for a reversing motor, utilizing instead a magnetic solenoid electrically interlocked so that the 180° rotation of the tubes can be brought about entirely by the same. The centrifuge **20'** incorporates a motor **22'** and a head **24'.** The motor **22'** drives the shaft **32'** by means of reduction gears **72** and **74.** The shaft **32'** carries a pair of spaced discs **76** and **76'** which are secured thereto for rotation therewith. A helical splined portion **78** is likewise fixed to the shaft **17** to rotate therewith. A spur wheel **80** engages with the splined portion **78** of the shaft **17** so that longitudinal movement of the spur wheel **80** with respect to the splined portion **78,** causes the spur wheel **80** to rotate with respect to the shaft **17** by an amount sufficient to rotate pinion wheels **48'** through 180°. The pinion wheels **48'** each carries a chuck or holder **46'** into which the lower end of a microhematocrit tube **16** can be inserted. Aligned openings **82** are provided in disc **76'** so as to support the microhematocrit tube **16** in vertical orientation, parallel to the rotational axis of shaft **17.**

The lower face of spur wheel **80** carries a thrust race **84** which can be urged upwards by means of the lever **86** mounted for vertical pivotable movement about shaft **88.** The thrust race **84** comprises a receptor ring **90** secured to the lower face of spur wheel **80** and a lower ring **92** having protrusions **94** arranged for engagement with shallow recesses **96** formed in ring **90.** The end **98** of lever **86,** remote from the race **84,** is operated by means of solenoid **101.** When the solenoid **101** is not energized, the spur wheel **80** is urged downward by light, annular spring **102,** arranged disposed between disc **76** and the spur wheel **80.**

The assembly consisting of the shaft **32'** with its splined portion **78,** the discs **76** and **76'** and the pinions **48'** rotate together in suitable bearings (not shown) while the motor **22',** the solenoid **101** and the lever **86** remain stationary.

Energization of the solenoid will rotate the tubes **52** through 180° whether the motor **22'** is running or not so that to meet the requirements of the method set forth above, is necessary only electrically to interlock the operation of the solenoid **101** with the stopping and starting of the motor **22'** so that the required sequence is obtained. Engagement and disengagement of the thrust race **84,** which acts as a clutch, effects the selective rotation of the tubes **16** along their own vertical axes, which, of course, lies offset from the axis of rotation of the centrifuge **20'.**

A further modified embodiment of the centrifuge apparatus according to the invention is illustrated in FIG. **7** and designated by reference character **100.** Apparatus **100** as described herein particularly is adapted to practice the method of the invention where the cycle utilized comprises four cycles of **45** second duration applications of greater than gravity force laterally to substantially vertically arranged columns of whole blood with means provided to effect limited rotation of each column about its own axis between each force application by reversal of the direction of rotation of the centrifuge head after each cycle.

The centrifuge apparatus **100** includes a housing **102,** including a troughlike portion **104** and a cover **106.** Wall **108** of housing **102** carries exterior accessible switch levers **110** and **112** for activating the power and buzzer means respectively which will be described hereinafter. Indicator lights **114** and **116** likewise are provided. Start switch **118** for initiating each test operation is provided.

The electrical control components of apparatus **100** are mounted within the troughlike portion while the head, centrifuge **120,** drive means **122,** and the timing means **124** are mounted on the cover portion, the centrifuge head being removably mounted to the protruding portion of the motor drive shaft **154** of means **122.** The drive means **122** and timer means **124** are mounted to be enclosed within the housing **102** when the cover **106** is engaged onto the portion **104.**

The centrifuge head **120** comprises a spool formed by mounting a spinner plate **126** fixedly secured to the shaft **128** for rotation therewith, and mounting a disc **130** to the upper end of said shaft **128** with the disc **130** arranged coaxial with said spinner plate **126** and being rotatable therewith. The shaft **128** is secured to the motor drive shaft **154** as by suitable means such as set screw **127.**

Gear support means **132** is arranged secured to the cover **106** and includes a collar portion **192** having a flat upper surface **192',** and a circumferential flange portion which is fastened to the cover **106.** The spur gear **134** has a circumferential ring portion **134'** carrying circumferential teeth **134''** and a central disc portion **135** to which it is fixedly mounted and by which the spur gear is mounted for independent rotation about the shaft **128,** that is, independent of rotation of the spinner plate **126.** A coating or film of thin machine oil or vacuum pump oil is applied to the surface **192'** of collar portion **192** to provide a friction drag upon the disc portion **135** which rests thereupon, and which, of course is transferred to the ring gear **134** and thereby is applied directly to the spinner plate **126.**

Tube holders **136,** similar to holders **46',** are mounted on spinner plate **126** for movement therewith about the axis of shaft **128.** The holders are spaced circumferentially substantially equidistant one relative to the other closely adjacent the peripheral edge of the spinner plate **126.** Each tube holder **136** has a top opening cavity **137** defined therein to receive the lower end of sample tube **150** and has resilient means for gripping said tube seated therein, such as O-ring **139.** Each holder is mounted on the upper end of a shaft **138**

which extends through suitable openings formed in said plate **126.** Pinion gears **140** fixedly are secured to the opposite ends of each shaft **138** thereby mounting the holders **136** on plate **126.** The holders **136** are rotatable with rotation of gears **140.**

A spur gear in the form of ring gear **134** is arranged so that its teeth **134''** are meshed with plural pinion gears **140.** Thus, rotation of the plate **126** will effect rotation of gears **140** while the ring gear **134** remains stationary, rotating holders **136** about their own vertical axes independent of the rotation of the shaft **128.** Limit means in the form of the upstanding pin **142** secured to the support means **132** and movable within the limit slot **144** formed in the spinner plate **126** is provided to limit the independence of movement of the spinner plate **126** and gear **134,** thereby limiting the degree of rotation of the gears **140** about their own axes. The limit means described may be said to comprise a lost motion coupling between the plate **126** and gear **134.**

The disc **130** has a plurality of bottom opening recesses **146** formed equispaced about the peripheral edge thereof and arranged in alignment with the axes of the holders **136** but slightly offset inwardly therefrom so that one end of the sample tube **150** can be seated within cavity **137** of holder **136** and the upper end retained within the respectively matching recess **146** to position the tube substantially vertically arranged but canted inwardly at its upper end toward the shaft **128.** Thus, when properly seated, tubes **150** are disposed, canted inwardly from true vertical between 3° and 6°, preferably by 3° and generally not more than about 6°. In this disposition, the tendency for the contents of the tube to be flung outward during the application of higher than gravity force on rotation or spinning of the centrifuge head **120** materially is reduced.

Motor mount **152** is secured to the undersurface of cover **106** with the drive shaft **154** thereof protruding through a suitable opening formed in said cover **106.** The drive means **122** for the apparatus **100** is supplied by a 400 RPM, 60 HZ, 115 volt AC reversible direction motor **156.** Here, motor **156** causes centrifugal force between 6G and 8 G to be applied laterally to the tubes **150** during the spin of header **120.** The particular size and RPM drive motor selected determines the centrifugal force exerted on the tubes **150,** and thereby is an important factor in selection of the duration of greater than gravity application cycle and program.

The method of the invention requires application of the greater than gravity force laterally and periodically to the sample in the tube i.e., the sample tube **150** and the column of blood therein. The duration of each cycle generally can be selected to provide results correlative with specifically known blood sedimentation methods.

With apparatus **100,** timer means **124** is provided to effect rotation of the spinner plate automatically through a sequence of four cycles of 45 seconds duration with the reversal of direction and rotation of the columns 180° about their own axes between each application of centrifugal force.

In the apparatus **100** illustrated, the timer motor **156** is an AC 60 cycle, 110 volt motor delivering 20 RPH.

The timing means **124** operates switch means, **184,186** which operates relays **184''** and **186''** automatically taking the sample columns through the selected test program.

A timing means **124** comprises a timer motor **158** mounted on platform **159,** which in turn is secured suspended below the cover **106** by means of bolts **160** and spacers **162.** The resultant drive shaft **164** of motor **158** is passed through a suitable opening in the platform **159** and wheel gear **166** is mounted at the free end thereof for rotation therewith. A second wheel gear **168** is coupled to gear **166** and is driven thereby. Gear **168** is fixedly secured to shaft **170** for rotation therewith. One end of shaft **170** is seated in journal **172** and the other end carries timer disc **176.** Timer disc **176** is secured to shaft **170** and continuously rotates therewith so long as timer motor **156** operates through the complete test program. The timer disc **176** has three paddle assemblies **178, 178'** and **178''** mounted thereto about the periphery thereof with the paddles **180, 180'** and **180''** extending outwardly from the circumferential edge thereof in vertical planes normal to the axis of shaft **170.** As illustrated, disc **176** is rotatable in the direction of arrow **177** with the paddle assemblies **178, 178'** and **178''** fixed in an equispaced series along said path. An upstanding pin **182** is secured normal to the disc **176** and rotates therewith. The paddle assemblies **178, 178'** and **178'',** when considering the direction of rotation of the disc, can be said to be substantially equispaced one relative to the others with paddle assemblies **180** and **180''** being disposed 180° apart. A pair of push-button activated switches **184** and **186** are arranged with their actuators **184'** and **186'** mounted to suitable bracket means (not shown) secured to the platform **159** so that the actuator **184'** of switch **184** is arranged in the path of travel of the paddles **180, 180'** and **180''** of paddle assemblies **178, 178'** and **178''** whereby each respective paddle can engage and depress said actuator **184'** by engaging same during passage therepast during rotation of the disc **176.**

The actuator **186'** of switch **186** is positioned to intercept the pin **182** whereby the continuing rotation of disc **176** causes pin **182** first to bear against actuator **186'** to depress same. On passing of said pin **182** past actuator **186',** said actuator returns to its normal condition. The switch **184** is connected to relay assembly **184''** which is electrically coupled to the reversible synchronous drive motor **156** to cause reversing of the direction of said motor each time the actuator **184'** is depressed. The switch **186** is connected to relay assembly **186''** operatively coupled electrically to both the drive motor **156** and to the buzzer means **190.** Depression of the actuator **186'** energizes the buzzer **190** and release of the actuator **186'** from engagement with the pin **182,** causes de-energization of the drive or spinner motor **156.**

A friction or other drag is applied to the centrifuge head **120** so that application of braking force to the motor **156** on de-energization of the same, causes a braking force to be applied directly to the head. Accordingly, the tubes **150** and the columns of test samples therein will be prevented from being rotated about their own vertical axes at least until the centrifuge head **120** starts up after coming to a substantially full stop, however momentary.

The friction drag described may be applied by means of the engagement of the collar portion **192** of gear support means **132** with the facing surface of gear **134** and the provision of a coating or film of light machine

oil sandwiched therebetween. Instead of the collar portion **192** being an upstanding ring integral with the support means **132,** it may take the form of a foam collar (not shown) secured thereto or even arranged coaxial about the shaft **128.** This oil interface friction drag arrangement is illustrated in detail in FIG. **12.**

An example in testing operation utilizing apparatus **100** now will be described. Samples of whole blood are taken and placed respectively in closed end, elongate tubes known as sedimentation tubes. The tubes are filled with sample to a predetermined level mark. The tubes containing the test samples are placed between the disc **130** and the spinner plate **126,** the lower ends of the tubes seated within the tube holders **136** while the upper ends are seated in the recesses **146** and held firmly by the resilient means **139.** The switch levers **110** and **112** are actuated respectively activating the apparatus **100.** The start toggle switch **118** is actuated initiating the test procedure and causing the spinner motor **156** to operate in one direction, say clockwise. Greater than gravity force in the range of 6.25 to 8 G is applied to the column of sample in each tube as the centrifuge head **120** is spun.

When motor **156** is energized to spin head **120,** motor **158** is energized simultaneously to rotate disc **176.** Timer disc **176** rotates to bring paddle **180** in contact with the actuator **184'**. Disc **176** continues to rotate so as to carry paddle **180** past said actuator **184'**. In passing, the paddle **180** depresses the actuator **184'**, causing the spinner motor **156** to reverse direction. This occurs 45 seconds after initiation of the spinner operation.

In reversing direction, the centrifuge head **120** comes to a momentary halt with the pin **142** at one end of the opening or slot **144.** The centrifuge head **120** then begins to rotate in the clockwise direction. The gear **134,** being mounted for free rotation about the shaft **128,** will remain stationary. The pinion gears **140** being mounted on the spinner plate **126,** and meshed with the gear **134,** will move along the circumference of now stationary gear **134** and will rotate about their respective axes until engagement of the pin **142** at the opposite end of the eccentric slot **144** will drive the gear **134** with the rotation of the spinner plate **126,** limiting the rotation of the pinion gears 140° to 180°. The rotation of the pinion gears **140** rotates the tube holders **136** and with same, the tubes **150** and the column of blood sample will be rotated. The braking must be gradual and not abrupt so that separation of the column from the inner tube wall will not occur. This is accomplished by the friction drag applied to plate **126.** The column must rotate with the tube wall.

The spinner motor **156** operates to drive the centrifuge head **120** in a clockwise direction for the next cycle of 45 seconds. At the elapse of 45 seconds, the next paddle **180'** will have brought around to depress the actuator **184'** and cause a second reversal of the spinner motor **156.** The spinner plate **126** again is brought to a momentary halt, and, in reversing direction, first moves relative to the gear **134** to bring the pin now at the other end of the slot **144,** back to the first, or now opposite end of said slot **144.** The pinion gears **140** have thus been rotated 180° about their own axes before any appreciable centrifugal force has been generated.

On completion of the movement of the pin **142** in the slot **144,** and engagement of said pin **142** with the spinner plate **126,** the spinner plate and the gear **134** are locked for rotation together, now in the counterclockwise direction for another **45** seconds until the spinner motor direction is reversed by engagement of the paddle **180''** against the actuator **184'** of switch **184** depressing same. The pinion gears **140** and hence, the holders **136,** tubes **150** and test sample columns therein, again are rotated about their own axes between applications of centrifugal force.

Coupled rotation of the spinner plate **126** and gear **134** is resumed for another and final 45 second interval. The timer plate **176** is continuously rotating during these last described operations, and, accordingly, continues to rotate. Approximately 45 seconds after the last-mentioned motor reversal, the pin **182** is brought into contact with the actuator **186'** by the continued rotation of the timer disc **176,** the actuator **186'** is released from its depressed condition. Now, the motor **156** is de-energized and the centrifuge head is brought to a halt.

The tubes **150** with their now partially packed red cell layer, are each compared with the initial level and a ratio taken which is reflective of the sedimentation rate of the sample. It is possible then to subject the tubes and the samples therein to substantially greater G force, such as **100** G in a conventional centrifuge so as to fully pack the red cells. The ratio of the fully packed cell level to the partially packed level is taken. This ratio, the resultant sedimentation rate taken to provide what can be described as a "Zeta Sedimentation Ratio," is independent of the effect of hematocrit and is related to the state of health of the source individual. The term "Zeta" refers to the "Zeta" potential between cells. The "Zeta" potential to which reference is made is effected by the concentration of asymmetrical protein molecules in the blood such as fibrinogen, gamma globuin, etc. The "Zeta Sedimentation Ratio" in a fashion analogous to the sedimentation rate has been found to be indicative of the state of health of the source individual. Unlike sedimentation rate determinations per se which measure rate of red cell fall, the value described here as the "Zeta Sedimentation Ratio" or ZSR provides a determination of the packing factor or closeness of packing of the cells. As a result, the ZSR is independent of the effect of "hematocrit," the relative quantity of red cells in the whole blood sample.

What it is desired to be secured by Letters Patent of the United States is:

1. A method for studying the sedimentation characteristics of whole blood comprising the steps of:

periodically applying greater than gravity force laterally to a generally vertically oriented column of test sample of whole blood in plural cycles of predetermined duration, rotating the vertically oriented column about its own long axis between each cycle, observing the level of the resulting packed portion of said column of test sample after completion of all said cycles and determining the relative concentration of cells in the packed and unpacked portions of said column.

2. The method as claimed in claim **1** wherein the greater than gravity force is applied to said column in a direction substantially normal to the long axis of said column.

3. The method as claimed in claim **1** in which the column is defined within an elongate tube of capillary pro-

portions and the rotation of the tube about its own long axis is performed so that the column rotates with the tube wall.

4. The method as claimed in claim **1** in which at least one of said cycles is of substantially greater duration than the others.

5. The method as claimed in claim **1** wherein the column of test sample is rotated 180° about its own long axis.

6. The method as claimed in claim **1** wherein the rotation of the column occurs only when there is less than one G force acting laterally relative to the column.

7. The method as claimed in claim **1** wherein the test column is translated along a circular path at a rate sufficient to apply a centrifugal force greater than one G laterally to the test column in periodic serial intervals of predetermined duration and the test column is rotated about its own axis between said intervals only when said centrifugal force acting laterally is less than one G.

8. The method as claimed in claim **7** wherein the rotation of the test column about its own axis is effected by reversal of the direction in which the test column is translated along said circular path.

9. The method as claimed in claim **8** wherein the rotation of the test column about its own axis occurs on initiation of the change of direction of translation of said test column.

10. The method as claimed in claim **1** and the additional step of fully packing the column of test sample on completion of all the said cycles by applying greater than **100** G force to the test column after all the cycles are completed and once the determination of concentration of cells in the initially packed portion has been completed.

11. The method as claimed in claim **1** wherein said determination is made by observation of the level of packed cells on completion of all the cycles and comparison of such level to the initial level of said test sample to provide a first packing factor.

12. The method as claimed in claim **11** and the additional steps of applying high G force to the test sample column subsequent to completion of said comparison to pack same to a maximum extent, thereafter comparing the final level of packed cells to the level of the cycled column to obtain a second packing factor and then comparing the pair of packing factors to provide a ratio.

13. The method as claimed in claim **1** in which the greater than gravity force is applied in at least two cycles to accelerate the formation of rouleaux in the sample column, next in a third cycle of a duration at least twice the duration of each of the first two cycles whereby to cause the formed rouleaux and the plasma components to migrate one through the other with the formed rouleaux assuming a thin layer on one side of the column, lastly applying at least one cycle of greater than gravity force for the same duration as each of the first two cycles.

14. The method as claimed in claim **13** in which each cycle is separated by rotation of the test column 180° about its own axis only when the force applied laterally to the column is less than one G.

15. The method as claimed in claim **1** in which the greater than gravity force is applied in at least two applications of a first duration to accelerate the formation of rouleaux in the sample column, next in a third application having a duration approximately three times longer than each of the first two applications whereby to cause the formed rouleaux and the plasma components to migrate one through the other with the formed rouleaux assuming a thin layer on one side of the column, applying at least one application of greater than gravity force for the same duration as each of said first two applications, the column being brought to rest so that the rouleaux layer is permitted to fall to the bottom of the column for observation of level.

16. The method as claimed in claim **1** in which the applied force is of the range of from 2 to 12 G.

17. The method as claimed in claim **1** in which the applied force is from 5 to 9 G.

18. The method as claimed in claim **1** in which the applied force is from 6 to 8 G.

19. The method as claimed in claim **1** in which said column is defined by filling an elongate tube with test sample to a predetermined level, orienting said filled tube in a centrifuge head with its long axis substantially parallel to the axis of rotation of the centrifuge head and rotating said tube in a circumferential path about said rotational axis of the centrifuge head during each cycle.

20. The method as claimed in claim **19** in which the greater than gravity force is applied to the column inclined at an angle theta from the vertical such that the product of the relative centrifugal force applied and the tangent of the angle theta is a value no higher than **1.**

21. The method as claimed in claim **19** in which the column is rotated first in at least two separated but successive cycles of predetermined duration, each centrifugation cycle being separated by a condition under which the column is under less than one G force acting laterally thereto, the column being rotated 180° about its own long axis only during the said condition subsequent to reaching said condition.

22. The method as claimed in claim **21** in which the rotation of the column is effected by reversing the direction of rotation of the centrifuge head.

23. The method as claimed in claim **1** in which a sample of whole blood is deposited in an elongate tube to define the column, the tube is placed in a centrifuge oriented substantially parallel to the rotational axis thereof, the column being rotated circumferentially about the centrifuge axis to apply said greater than gravity force, said circumferential rotation occurring in cycles of predetermined duration, each circumferential rotation being separated by a period when the centrifuge is brought to a substantially at rest condition, and the column is rotated about its own axis only subsequent to reaching said substantially at rest condition.

24. The method as claimed in claim **23** in which the duration of the cycles is inversely prportional to the G force generated by the centrifuge and applied to the column.

25. The method as claimed in claim **24** in which the orientation of the test column is no greater than an angle of 6° from vertical orientation.

* * * * *

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UNITED STATES PATENT OFFICE
CERTIFICATE OF CORRECTION

Patent No. 3,824,841 **Dated** July 23, 1974

Inventor(s) BRIAN S. BULL

It is certified that error appears in the above-identified patent and that said Letters Patent are hereby corrected as shown below:

Column 1, line 15, after "improved" insert --method--; column 3, line 54, change "is" to --in--; column 7, line 32, change "comapred" to -compared--; column 15, line 46, 140° should be --140-- and in heavy type.

Signed and sealed this 29th day of October 1974.

(SEAL)
Attest:

McCOY M. GIBSON JR.
Attesting Officer

C. MARSHALL DANN
Commissioner of Patents