

[54] METHOD OF AND APPARATUS FOR
CHEMICALLY SEPARATING PLASMA OR
SERUM FROM FORMED ELEMENTS OF
BLOOD

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[63] Continuation-in-part of Ser. No. 207,577, Dec. 13,
1971, abandoned, which is a continuation of Ser. No.
847,469, Aug. 4, 1969, abandoned.

[52] U.S. Cl. 195/1; 195/103.5 R; 23/230 B;
195/127

[51] Int. Cl. G01n 33/16

[58] Field of Search 195/1, 103.5 R, 127;
23/258.5, 230 B; 210/538

[56] **References Cited**
FOREIGN PATENTS OR APPLICATIONS

969,741 9/1964 United Kingdom..... 195/1

OTHER PUBLICATIONS

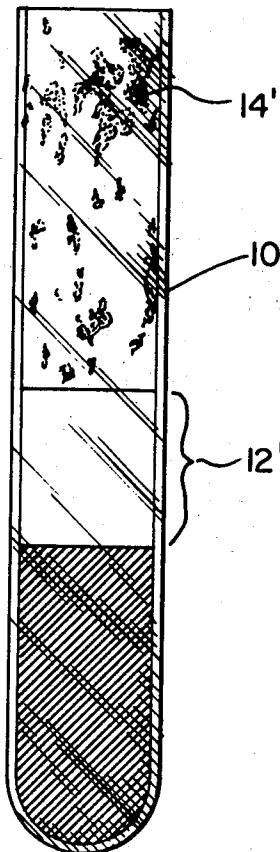
August, A., Am. J. Med. Tech., Vol. 31, pp. 271-278,
(1965).

Primary Examiner—Alvin E. Tanenholtz
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Kurtz & Mackiewicz

[57] **ABSTRACT**

A process for separating plasma or serum from formed
elements of the blood by adding a positively charged
polymer and a lectin to a tube into which blood is
drawn to effect precipitation of the formed elements
of the plasma or serum.

15 Claims, 9 Drawing Figures



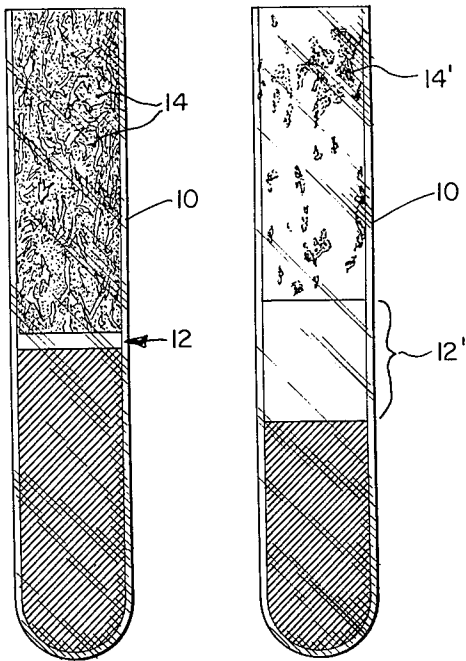


Fig. 1a **Fig. 1b**

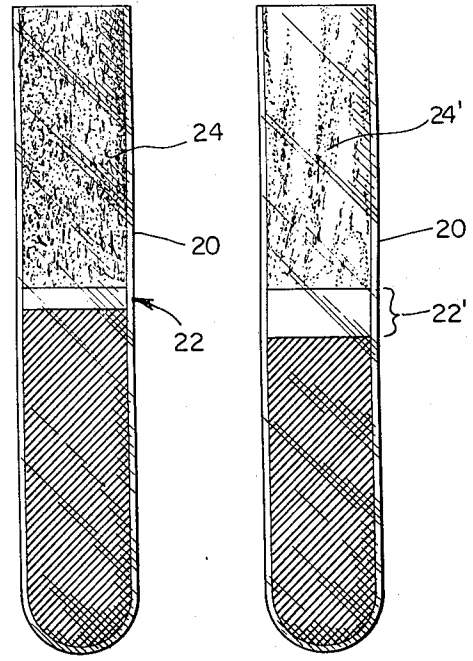


Fig. 2a **Fig. 2b**

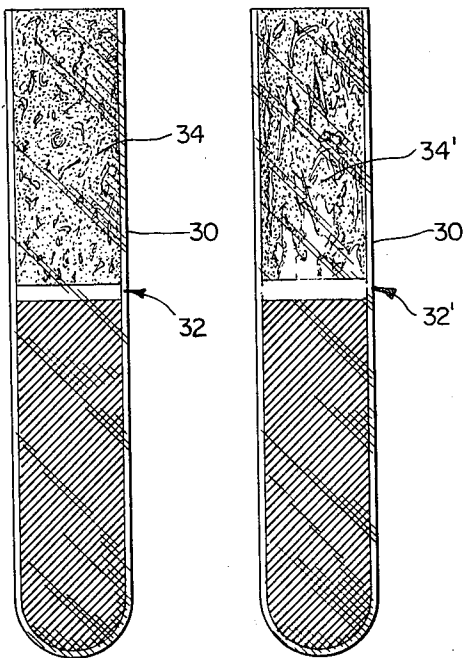


Fig. 3a **Fig. 3b**

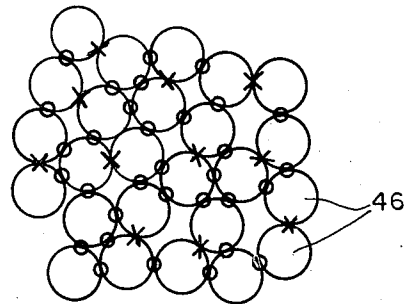


Fig. 4

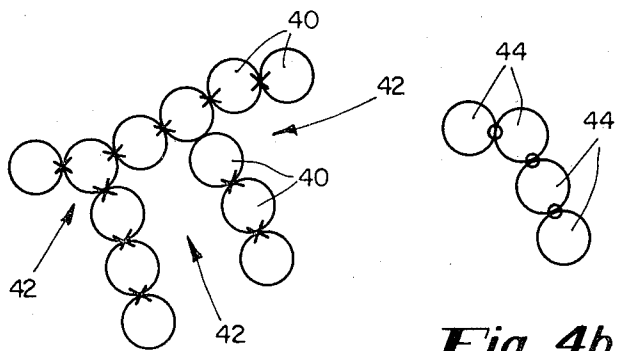


Fig. 4a

Fig. 4b

METHOD OF AND APPARATUS FOR CHEMICALLY SEPARATING PLASMA OR SERUM FROM FORMED ELEMENTS OF BLOOD

RELATED APPLICATIONS

This is a continuation-in-part of application Ser. No. 207,577 filed Dec. 13, 1971, now abandoned which is a continuation of application Ser. No. 847,469 filed Aug. 4, 1969 and now abandoned.

BACKGROUND OF THE INVENTION

This invention relates to the separation of formed elements of the blood from plasma or serum.

At the present time, the separation of formed blood elements, e.g., red blood cells, from plasma or serum is accomplished almost entirely by mechanical separation techniques. Of the various mechanical separation techniques, centrifuging is most popular. However, mechanical separation techniques including centrifuging are cumbersome in that they require rather elaborate mechanical apparatus. Moreover, mechanical separation techniques may provide somewhat less than optimum plasma or serum yields. In addition, mechanical separation techniques can result in faulty plasma or serum analysis due to the time involved in transporting a blood sample to a centrifuging apparatus thereby delaying the separation of plasma or serum from the formed elements of the blood.

Ideally, separation of the cells from the plasma or serum should take place immediately thereby decreasing the amount of serum or plasma contamination by cell breakdown.

Chemically assisted mechanical separation techniques have been explored. One such technique, which is disclosed in British Pat. No. 969,741 — Brewer, involves the use of "agglutination, coagulation or precipitation" of solid components of blood due to the presence of a phytohemagglutinin and mechanical stirring.

It has been found that the phytohemagglutinin alone, in the absence of stirring, will result in very slow precipitation of the blood cells which is believed to be due to the fact that clumps of blood cells which are formed are small and not particularly dense. Even with stirring, it is believed that any separation of serum from blood cells is too slow to be clinically feasible. More importantly, the stirring of the blood sample will itself cause cell breakdown and contaminate the results of serum or plasma analysis.

The use of "lectins" as hemagglutinins was described by Irvin E. Liener in a paper presented for discussion at the Seed Protein Conference sponsored by the Southern Utilization Research and Development Division, Agricultural Research Service, United States Department of Agriculture, New Orleans, La., Jan. 21-24, 1963 and printed in *Economic Botany* as an article entitled "Seed Hemagglutinins". In this paper, there is considerable discussion of a theorized agglutinating mechanism involving receptor sites on the blood cells to which the lectin attaches. In this manner, two blood cells may be attached or clumped where their receptor sites are linked by the lectin.

Another chemically assisted mechanical separation technique has involved the use of an agglutinating agent comprising a positively charged polymer such as Polybrene (hexadimethrine bromide). Such a use of Polybrene is disclosed in an article entitled "A New Technic for Separation of Human Leukocytes" by Par-

vitz Lalezari, appearing in *Blood*, vol. 19, No. 1, January, 1962 where the blood sample containing cells agglutinated by the Polybrene is centrifuged to effect precipitation. In an article by Anne August, appearing in the *American Journal of Medical Technology*, "The Effect of Antiheparin Drugs on Human Blood", vol. 31, No. 4, July-August, 1965, Polybrene is also discussed as a means for inducing agglutination of blood cells.

Precipitation of the blood agglutinated with Polybrene into fairly large clumps is very slow without centrifuging. Moreover, the total amount of plasma or serum which may be obtained with Polybrene is relatively small. As a result, the total volume of serum which may be obtained in a given length of time is small as compared with the volume obtained utilizing the phytohemagglutinin described by Brewer. Moreover, the longer the blood cells remain in the plasma, the greater the contamination of the plasma so as to adversely affect the plasma analysis. In addition, a good deal of debris will remain in suspension in the plasma after precipitation. Any attempt to increase the concentration of Polybrene beyond a certain point for speeding precipitation will result in lysis of the blood cells. The reason for this lysis is not completely understood although it is believed that large concentrations of the positively charged polymer may result in collisions between the polymer and the cells which rupture the membrane of the cells. It is also possible that the large concentration of the positively charged polymer creates such substantial deformation of the cells as described by A. Katchalsky et al. in *Biochimica Et Biophysica Acta*, "Basic Polyelectrolytes in Red Blood Cells", vol. 33, pp. 120-139, 1959, that the cells actually rupture.

SUMMARY OF THE INVENTION

It is a general object of this invention to provide an improved method of chemically precipitating formed blood elements from plasma or serum.

It is a further object of this invention to employ a chemical separation technique which will not adversely effect the analysis of the plasma or serum.

It is a further object of this invention to employ a chemical separation technique which assures separation of the formed elements of the blood from the serum or plasma within a short period of time.

It is also an object of this invention to employ a chemical separation technique which substantially prevents contamination of the plasma or serum.

It is still a further object of this invention to employ a chemical separation technique which yields substantial amounts of plasma or serum which has been separated from the formed elements of the blood.

In accordance with these and other objects, two chemical reagents are utilized to cause agglutination of the formed elements of the blood and precipitation of these elements from the plasma or serum of the blood. The reagents comprise a positively charged polymer (basic polyelectrolyte) such as Polybrene, and a lectin (hemagglutinin plant extract), such as Bacto Phytohemagglutinin P, which are added to a blood collection tube either during the manufacturing of the tube or at a later time by, for example, conventional needle insertion techniques. The blood taken from the donor is received within the tube and the plasma is separated out as a result of the action of the above-mentioned chemi-

cals with the blood. The separation mechanism occurs spontaneously within one-half hour or less.

In accordance with one important aspect of the invention, the chemical reagents create large three dimensional dense clumps of blood cells which readily precipitate to the bottom of the collection tube. These clumps are desirable in that they are not easily broken apart by jarring the blood sample.

One explanation for the density, three-dimensionality and durability of these clumps, which is believed to be correct, is that a lectin has an increased agglutinating ability in the presence of a positively charged polymer. Since the formed elements of the blood are all negatively charged, they tend to repel one another and the probability of the lectin attached to a receptor site on one cell coming into contact with a receptor site of another cell to form a clump is lowered by this mutual repulsion. However, when the positively charged polymer is added to the blood sample and attracted to the negatively charged cells, the cells and polymer form neutralized masses which permit the lectin to more effectively agglutinate the blood cells since the cells are more likely to come into sufficiently close contact to permit the lectin to form links between the cells at receptor sites and cells within a clump are able to move into a densely packed configuration due to the absence of repulsion.

In addition to the neutralizing effect which allows the lectin to more effectively perform its agglutinating function, the positively charged polymer also provides its own agglutinating function. In other words, the single cell within the clump may be linked to one or more cells by the lectin while the same cell is linked to one or more other cells by the positively charged polymer. As a result of utilizing the two different types agglutinating agglutinating agents, each cell is able to be attached to a greater number of cells than would be the case if only one of the two different types of agglutinating agents were utilized. This agglutinating mechanism also helps create increased density of cells in a clump and larger three dimensional clumps.

The large three dimensional clumps are essentially large net-like structures which are capable of dragging debris and fibrin down through the plasma. As the clumps collect at the bottom of the collection tube, a substantially integral structure is formed wherein all of the individual clumps are connected to one another.

Other objects and a fuller understanding of the invention will be had by referring to the following description in conjunction with the drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1a is an elevational view of a container immediately after blood, lectin and a positively charged polymer have been combined in accordance with this invention;

FIG. 1b is an elevational view of the container and the contents of FIG. 1a approximately 15 minutes later;

FIG. 2a is an elevational view of a container immediately after blood and a lectin have been combined;

FIG. 2b is an elevational view of the container and the contents of FIG. 2a approximately 15 minutes later;

FIG. 3a is an elevational view of a container immediately after blood and a positively charged polymer have been combined;

FIG. 3b is an elevational view of the container of FIG. 3a approximately 15 minutes later;

FIG. 4 is a schematic illustration of a clump of blood cells agglutinated in accordance with this invention;

FIG. 4a is a schematic illustration of a clump of blood cells agglutinated with a positively charged polymer; and

FIG. 4b is a schematic illustration of a clump of blood cells agglutinated with an organic hemagglutinin.

DESCRIPTION OF THE PREFERRED EMBODIMENT

In the preferred embodiment of my invention, 5 milligrams (5mgs.) of Polybrene, a positively charged polymer (hexadimethrine bromide), which is manufactured by Abbott Laboratories and 0.05cc of Bacto Phytohemagglutinin P., a lectin (hemagglutinin plant extract) manufactured by Difco Company, are added to a 10cc Becton-Dickinson red top vacutainer (this tube initially contains no chemical reagents). The Bacto Phytohemagglutinin P. is manufactured in 5cc vials and is assayed according to potency, not weight. It is understood that other vacuum tubes may be utilized in practicing the present invention, and that the above-mentioned chemical reactants can be added during the manufacturing of the tube, or at a later time by, for example, conventional needle insertion techniques.

When blood which is taken from a patient or donor (approximately 9cc's of blood) enters the tube containing the positively charged polymer and the lectin, the formed blood elements are caused to separate from the plasma. This separation occurs due to the precipitation of large, dense, three dimensional clumps, which precipitation occurs within one-half hour or less so as to limit the contamination of the plasma or serum. One explanation for the size, density and three dimensionality of these clumps as compared with those obtained when lectin is utilized alone or Polybrene is utilized alone, is that the positively charged polymer Polybrene actually increases the agglutinating ability of the lectin. Since the formed elements of the blood are negatively charged, they tend to repel one another. The probability of the lectin attaching to a receptor site and one of the cells coming into contact with a receptor site of another cell to form a clump is lowered by this mutual repulsion. However, with the Polybrene being present, the negative cells are attracted to the Polybrene to create substantially electrically neutralized blood cell-polymer combinations. The lectin which attaches to the blood cell is then better able to agglutinate the blood cells and to clump since it does not have to overcome or otherwise work against the repulsion forces between the negatively charged blood cells since the blood cells have in effect been neutralized by the Polybrene. As a result, large, dense, three dimensional clumps of red blood cells are formed which rapidly precipitate to the bottom of the sample container. It is also important to note that the clumps of blood cells are very durable in that they do not break-up when the sample container is shaken, jarred or otherwise stirred. It is believed that this reflects the strength of the lectin agglutinating link between blood cells which no longer has to overcome the repulsion of the blood cells due to the neutralization by the Polybrene. The very dense nature of the clumps is to be attributable to the fact that the lectin is capable of upholding the blood cells in a closely linked relationship since the cells are no longer repelling one another again due to the neutralization by the positively charged polymer.

In order to enhance the precipitation of the formed elements of the blood, the long axis of the collection tube is placed parallel to the surface of a counter top. After the separation has taken place, the tube is gradually rotated manually to dislodge and put into solution any cells that remain on the glass part of the tube or stopper. A rotation of the tube also aids in filtering out the fibrin in the plasma to leave serum. The long axis of the tube is then placed perpendicular to the counter top.

When serum, rather than plasma, is desired, a substance can be added to inhibit the formation of fibrin or destroy the formation. A substance may also be added to cause clotting and precipitate the fibrin once it is present. For example, Fibrinolysin may be added to the collection tube to prevent the formation of fibrin, or a clotting agent such as thrombin may be added to accelerate clotting of the fibrin. The clumped blood cells which form a net-like structure effectively drag the fibrin downwardly with the clump of formed blood elements to in effect filter, at least to some degree, the fibrin out of the plasma.

In accordance with this one very important aspect of this invention, the use of a positively charged polymer in combination with a lectin produces a very rapid precipitation of the formed elements of the blood as compared with the rate of precipitation achieved using a lectin alone or a positively charged polymer alone. FIG. 1a shows a collection tube filled with a blood sample substantially immediately after the blood sample has been combined with Polybrene and Bacto Phytohemagglutinin P. Note that the formed elements of the blood near the bottom of the tube 10 have begun to precipitate as can be seen from the light region 12 which represents plasma and a relatively low concentration of the formed blood elements. Precipitation results from the clumping of the formed blood elements, which clumps 14 have been formed on the wall of the tube 10. Microscopic examination of the clumps 14 indicates that they are substantially three dimensional structures comprising densely packed blood cells. After 15 minutes have passed, a layer 12' of plasma is achieved about 99% of the precipitated formed elements of the blood. In this interim, a substantial number of the clumps on the wall of the tube 10 have slid down the wall through the plasma 12' and into the precipitated elements at the bottom of the tube 10. Note that the clumps 14' which remain on the wall of the tube 10 are much larger than the clumps 14 which existed immediately after the blood had been combined with the Polybrene and the Bacto Phytohemagglutinin P.

FIG. 2a is a tube 20 filled with a blood sample immediately after it has been combined with Bacto Phytohemagglutinin P. alone without the Polybrene being present. Once again, a layer of plasma 22 has begun to form near the top of the blood sample due to the immediate precipitation within the sample as a result of the clumps of blood elements which are created by the Bacto Phytohemagglutinin P. The clumps 24 on the walls of the tube 20 indicate that the Bacto Phytohemagglutinin P. produces much smaller clumps than those produced by the combination of the Polybrene and the Bacto Phytohemagglutinin P. Note that the rate of precipitation of the formed blood elements in the blood sample is much slower as depicted in FIG. 2b. The layer of plasma 22' above 99% of the formed blood elements

at the end of 15 minutes of precipitation has approximately half the height and volume as that produced with the combination of Bacto Phytohemagglutinin P. and Polybrene as depicted in FIG. 1b. Note also that a very large number of clumps 24' remain on the walls of the tube 20 after 15 minutes of precipitation. Furthermore, these clumps are of substantially the same size of the clumps 24 shown in FIG. 2a. In other words, the size of the clumps does not increase with time and the clumps do not become large enough to slide under the influence of gravity down the walls of the tube 20.

In a container 30 illustrated in FIG. 3a, Polybrene has just been added to a blood sample and precipitation has begun due to the agglutinating properties of the Polybrene which forms clumps 34 on the wall of the container 30. However, after 15 minutes, very little actual precipitation has occurred as shown in FIG. 3b wherein a very shallow layer of plasma 32' has formed above 99% of the blood cells. Note that this layer of plasma 32' formed by the Polybrene alone is much more shallow than the layer of plasma 22'. This is the case even though the initial clumps 34 and the subsequently formed clumps 34' which are quite fragile are larger than the clumps 24 and 24' which are formed with the use of the Bacto Phytohemagglutinin P. alone. Thus, the size of the clumps alone is not the key to precipitation of the blood cells to maximize the amount of plasma which can be obtained in a given amount of time.

Note further that the amount of plasma 12' obtained utilizing the combined agglutinating substances of the invention as shown in FIG. 1b is much more than the sum of the plasma obtained from utilizing either of those agglutinating agents alone as shown in FIGS. 2b and 3b. Actual measurements indicate that the amount of plasma 12' obtained utilizing this invention is approximately twice the amount of plasma obtained in utilizing the individual ingredients alone as depicted in FIGS. 2b and 3b. In other words, the result achieved in utilizing the combination of the Polybrene and the Bacto Phytohemagglutinin P. is more than (approximately twice) the sum of the results achieved when utilizing these two ingredients separately.

This synergistic result indicates that the positively charged polymer and the lectin are interacting with one another to increase the rate of precipitation of the clumped blood cells. If the polyelectrolyte and the organic lectin were not interacting but merely performing their own, independent agglutinating functions, the amount of plasma obtained in a given amount of time utilizing the combination should be equal to the sum of the plasma obtained when the polyelectrolyte and the organic hemagglutinin are utilized alone.

An explanation of this interaction, which is believed to be correct in view of analyses performed after observing the results of this combination, will now be set forth with reference to FIG. 4 in conjunction with FIGS. 4a and 4b. FIG. 4 depicts a fragile clump of blood cells 40 which are linked on to one another by polymeric seams schematically depicted by the symbol x. Note that each of the blood cells 40 is linked to at least one other blood cell by the polymeric seam x and those blood cells 40 in the middle of the clump are linked to two or three blood cells. However, each blood cell does have a substantial surface area which is not adjacent, in contact or connected to any other blood cell. An actual photograph of this type of polymeric

bonding between the blood cells 40 is shown on page 132 of the aforesaid Katchalsky et al article entitled "interactions of Basic Polyelectrolytes with the Red Blood Cell". Since each cell 40 is apparently capable of forming a link over a limited surface area, the resulting clump is not particularly dense and voids 42 between the interconnected cells 40 of clump clump do exist. The apparent inability of the blood cells to form polymeric bonds along more of the surface area of each blood cell is believed to be due to the fact that each negatively charged blood cell 40 is capable of being attracted to a limited number of positively charged polymers before the overall charge on the combination polymerblood cell is neutralized so as to prevent the attraction of that particular blood cell to any other positively charged polymers.

FIG. 4b depicts a somewhat smaller clump than that shown in FIG. 4a wherein the agglutination is achieved by a lectin. As shown in FIG. 4b, four cells 44 are linked or bonded as depicted by the symbol $\circ$. The nature of the link $\circ$ is not particularly well understood but one very plausible explanation has been offered in the aforesaid Liener article which suggests that each blood cell includes a limited number of receptor sites to which a hemagglutinin such as Bacto Phytohemagglutinin P. may be attached. Thus for two cells to attach themselves to one another utilizing an agglutinating agent such as Bacto Phytohemagglutinin P., receptor sites on two different cells, must come in close proximity with the lectin therebetween. Then the clumping of two cells 44 can occur. With this limitation on the ability of the cells 44 to clump, it is not surprising that each of the clumps 42 as depicted in FIGS. 2a and 2b is rather small particularly in view of the repulsion between negatively charged blood cells. It is also not surprising that large areas of each of the cells 44 are not liked with any other cells due to the absence or scarcity of receptor sites to form those links.

When the positively charged polymer utilized to form the clump depicted in FIG. 4a is combined with the lectin hemagglutinin which forms the clump depicted in FIG. 4b, a dramatic difference is observed in the nature of the clumps which are formed as depicted in FIG. 4. As previously mentioned, it is believed that the positively charged polymer is attracted to the negatively charged elements or cells so as to neutralize those cells and thereby permit the lectin which becomes attached to these cells to more effectively agglutinate these cells since the negative charge on the blood cells has been neutralized to preclude repulsion between these cells. As shown in FIG. 4, the large, dense clump of blood cells 46 is formed by a substantial number of individual blood cells which are completely surrounded by other blood cells and attached thereto by a plurality of lectin links $\circ$. Increased number of lectin links is, as previously mentioned, due to the presence of the positively charged polymer which neutralizes the negative charge on the cells to preclude or at least substantially reduce the repulsion therebetween. In addition, the positively charged polymer forms bonds or seams $\times$ between blood cells in the clump so as to perform the dual function of increasing the agglutinating ability of the lectin while also providing its own agglutinating function. When the clump in FIG. 4 is analyzed, it will be seen that a substantial number of the blood cells in the clump have both a lectin link $\circ$ and a polymer seam $\times$ with other blood cells.

As mentioned previously, the size of the clumps is not the only factor which determines the rate and extent of precipitation. If it were, the positively charged polymer alone would precipitate more rapidly than the organic lectin alone which would be inconsistent with the experimental results depicted in FIGS. 2b and 3b. In this connection, it is believed that the large, spider-like clumps of blood cells, an example of which is depicted in FIG. 4a, create a type of structure in the blood sample which is not only slow to precipitate but actually self-supporting due to the formation of fibrin so as to account for the rather shallow layer of plasma 32' which is obtained when utilizing the positively charged polymer alone as depicted in FIG. 3b. In contrast, the combination of the positively charged polymer and the lectin achieves a dense clump which precipitates rapidly and collapses into a rather limited area so as to obtain a rather deep layer of plasma 12' as depicted in FIG. 1b due to the inhibition of fibrin formation. This explanation is borne out by microscopic examination of the blood cells at the bottom of the tube 10 when the combination of the positively charged polymer and the lectin is utilized. In particular, the blood cells at the bottom of the tube 10 as depicted in FIG. 1b seems to be a substantially unitary or integral mass of compact blood cells which cling together when the tube 10 is tipped, shaken, jarred or otherwise disturbed. In contrast, clumps of blood cells precipitated to the bottom of containers 20 and 30 are not part of an integral mass and separate from one another when the tube is disturbed.

In accordance with another important aspect of the invention, rapid precipitation of the blood cells is achieved while preserving the integrity of the tests results and analysis of the plasma.

The chart reproduced below represents actual test results of my system (M.S.) compared with the presently used conventional centrifuged system (C.S.). A random selection of blood donors was used, and the blood samplings from the centrifuged system were obtained by using the standard Becton-Dickinson tubes and reagents. In my system, Polybrene and Bacto Phytohemagglutinin P. were put into a Becton-Dickinson red top vacutainer tube which initially contained no reagents. Approximately one-half hour to 3 hours after withdrawing the blood, the plasma from my tube and the standard tube was removed. Most of the tests were performed with the Technicon Auto-analyzer; the remainder being performed manually.

TEST	C.S.	M.S.	TEST	C.S.	M.S.
Glucose	96	99	Chloride	104	107
(C.S. -	122	126		100	103
Gray top	96	96		97	99
tube used)	111	112		109	112
	141	143		111	112
BUN	23	24	Sodium	133	137
	20	20		137	134
	16	15		139	138
	22	22		140	142
	21	22		137	136
	31	31			
Total	6.9	6.9	Potas-	4.3	4.2
Protein	6.1	6.5	sium	4.0	4.0
	7.0	7.2		3.0	2.9
	6.8	6.9		4.6	4.7
	6.7	6.5		4.2	4.4
Albumin	5.3	5.0	Calcium	10.4	10.3
	4.6	4.4		9.6	9.6
	4.8	4.7		9.8	9.8
	4.9	4.9		9.4	9.5

-Continued

TEST	C.S.	M.S.	TEST	C.S.	M.S.
SGPT	5.0	4.9	Phosphorus	9.0	9.1
	12	12		4.1	3.9
	8	9		3.3	3.2
	9	8		3.9	3.7
Cholesterol	24	26	Uric Acid	2.5	2.5
	238	228		6.9	6.9
	324	314		4.7	4.6
	267	257		10.6	10.3
CO ₂	299	289	Creatinine	5.6	5.5
	26.5	26.5		0.8	0.8
	27.5	26.0		0.8	0.8
	27.5	26.0		1.9	1.9
Alk. Phos.	23.0	22.5	T-4	1.4	1.4
	26.0	25.0		1.0	1.1
	5.8	5.6		6.1	6.2
	8.8	8.6		6.3	6.5
Serum Pregnancy Test	6.4	6.2	P.B.I.	7.1	7.1
	7.6	7.4		7.7	7.9
	6.6	6.2		6.8	6.9
	Positive	Positive		7.2	7.4
L.E. Latex	Negative	Negative	T-3	5.6	5.9
	Positive	Positive		1.12	1.00
	Negative	Negative		1.07	.98
	Negative	Negative		1.04	1.07
Heterophile	Positive	Positive	C. Reactive Protein	Negative	Negative
	Negative	Negative			
	Negative	Negative			
	Negative	Negative			
R. A. Latex Fixation	Positive	Positive	R. A. Latex Fixation	Negative	Negative
	Negative	Negative			
	Negative	Negative			
	Positive	Positive			

The results reproduced in the above chart clearly indicate the reliability of the method of my invention as compared with the standard centrifuged separation method or any other presently employed method.

In the foregoing, two specific chemical substances have been mentioned for use in combination in practicing the invention, namely, the positively charged polymer or basic polyelectrolyte Polybrene (hexadimethrine bromide) and the organic hemagglutinin, Bacto Phytohemagglutinin P. It will of course be appreciated that other substances may be utilized in practicing the invention. For example, another polyelectrolyte such as polylysine hydrobromide, polyornithine hydrochloride, polyarginine sulfate, polyvinyl amine hydrobromide and protamine sulfate may be utilized. Other lectins suitable for use in the invention include ricin (extract from the castor bean), Concanavalin A (globular type proteins isolated in crystal form from jack bean meal as described by J. D. Sumner, *Journal of Biological Chemistry*, vol. 37, page 137 (1919)) and soybean hemagglutinin.

In the case of Polybrene and Bacto Phytohemagglutinin P. specific concentrations have been described. These concentrations may vary but in general, it is preferred that a concentration be utilized which is equal to 50-95% of the concentration which will lyse the blood cells. This permits you to maximize the clumping and the precipitation without hemolysis. Thus it will be appreciated that the actual physical amounts of the different substances may vary. For example, ricin is a very powerful agglutinating agent and very small amounts of ricin can result in hemolysis. Also, it will be appreciated that the invention may be utilized with blood samples of various sizes in excess of 30 cubic millimeters.

Although a specific embodiment of the invention has been described and others have been suggested, it will

be understood that the invention embraces these and other embodiments as set forth in the appended claims.

What is claimed is:

1. A method of separating serum from the formed elements in a blood sample comprising the steps of:
 - a combining a lectin hemagglutinin, a positively charged polymer and a blood sample in a container;
 - b agglutinating the formed elements into clumps with said lectin and said positively charged polymer such that each of a plurality of formed elements in each clump are agglutinated with another formed element with a polymeric seam therebetween and agglutinated with a further formed element with a lectin link therebetween; and
 - c precipitating said clumps to the bottom of the container to create a layer of plasma or serum above said formed elements.
2. The method of claim 1 wherein said clumps of formed elements are combined to form a substantially integral mass after precipitating.
3. The method of claim 2 wherein said clumps drag fibrin downwardly therewith while precipitating.
4. A method of separating formed elements of the blood from serum comprising the steps of:
 - a combining a blood sample with a lectin hemagglutinin and a positively charged polymer in a container;
 - b agglutinating the formed elements of the blood with said hemagglutinin and said positively charged polymer; and
 - c precipitating the agglutinated formed elements of the blood so as to form a layer of serum above 99% of the formed elements of the blood in a given length of time, said layer having a total volume greater than the sum of the volume in a layer of serum above 99% of all elements in a blood sample of the same size combined with said lectin alone after the same given length of time plus the volume in a layer of serum above 99% of all elements of the blood in a blood sample of the same size combined with said positively charged polymer after the same given length of time.
5. A method of separating formed elements of the blood from serum comprising the steps of:
 - a combining a blood sample with a lectin hemagglutinin and a positively charged polymer;
 - b agglutinating the formed elements of the blood with said lectin and said positively charged polymer; and
 - c precipitating the agglutinated formed elements of the blood at a rate in excess of the sum of the rates of precipitation achieved with said lectin alone in said blood sample and said positively charged polymer in said blood sample.
6. The method of separating plasma from formed elements of blood comprising the steps of:
 - a collecting a sample of blood and placing the sample in a blood collection container; and
 - b reacting said blood sample with a lectin hemagglutinin and a positively charged polymer whereby the formed blood elements separate from the plasma.
7. The method according to claim 6 wherein said positively charged polymer is Polybrene.
8. The method according to claim 6 wherein said hemagglutinin is Bacto Phytohemagglutinin P.

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9. The method of claim 6 wherein the hemagglutinin and the positively charged polymer are simultaneously reacted with the blood.

10. The method according to claim 6 wherein said blood collection container is hollow cylindrical in configuration and has a longitudinal axis and comprising the additional step of disposing said blood collection container with its longitudinal axis in a substantially horizontal plane to enhance precipitation of the formed blood elements from the plasma.

11. The method according to claim 6 to produce serum, including the further step of adding a substance for inhibiting the formation of fibrin.

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12. The method according to claim 11 wherein said substance is thrombin.

13. In combination, a blood collection container containing a hemagglutinin and a positively charged polymer, said container having volumetric capacity sufficient to hold more than 30 cubic millimeters of blood.

14. The combination according to claim 13 wherein the container also contains a substance for inhibiting the formation of fibrin.

15. The combination of claim 13 wherein the said container is a vacuum type blood collection tube.

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UNITED STATES PATENT OFFICE
CERTIFICATE OF CORRECTION

Patent No. 3,902,964 Dated September 2, 1975

Inventor(s) Donald J. Greenspan

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

Column 3, line 36, the word "aggulatinating" is repeated twice.

Column 7, line 3, the word "interactions" should be capitalized;
line 7, after the word "clump" delete the word "clamp".

Signed and Sealed this

sixteenth Day of December 1975

[SEAL]

Attest:

RUTH C. MASON
Attesting Officer

C. MARSHALL DANN
Commissioner of Patents and Trademarks