Feb. 4, 1958 E. J. COHN 2,822,126

CONTINUOUS FEED CENTRIFUGE

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Fig. 1.

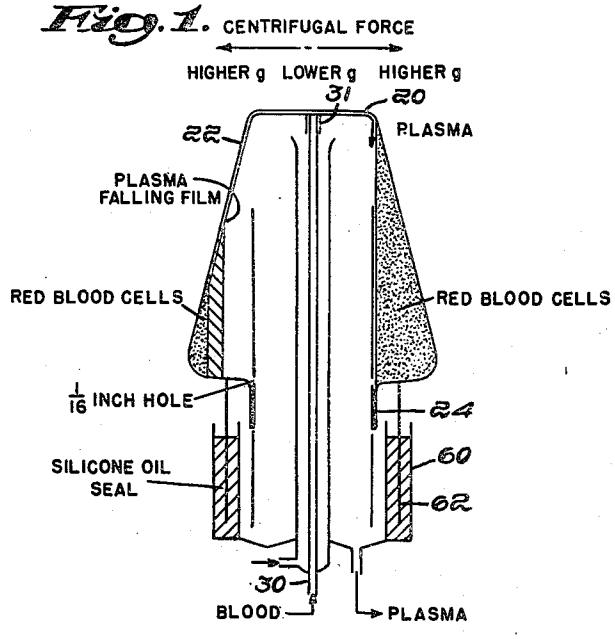
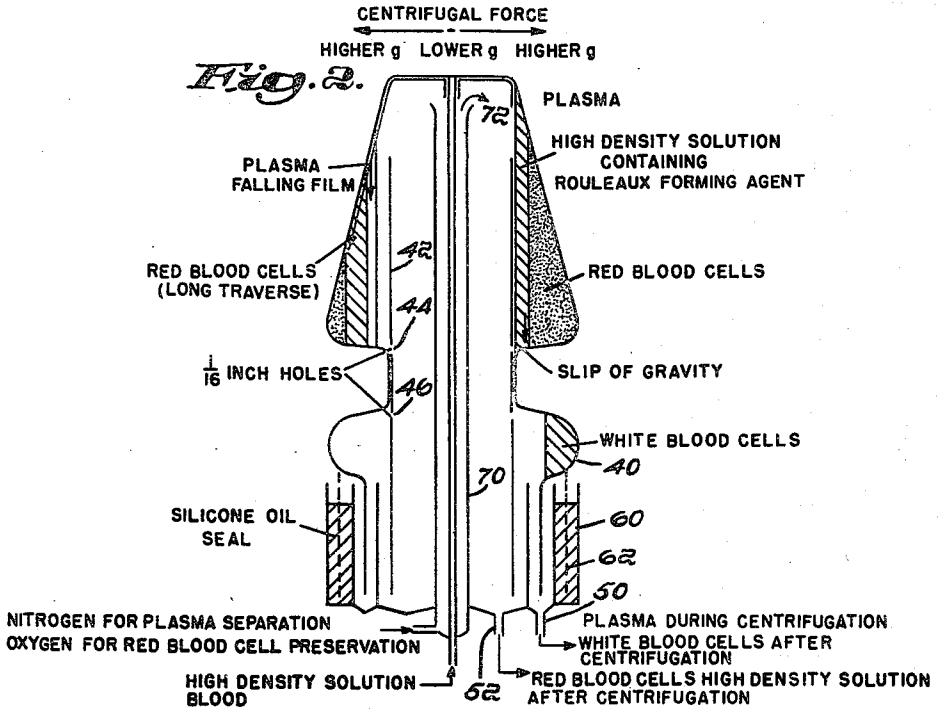


Fig. 2.



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CONTINUOUS FEED CENTRIFUGE

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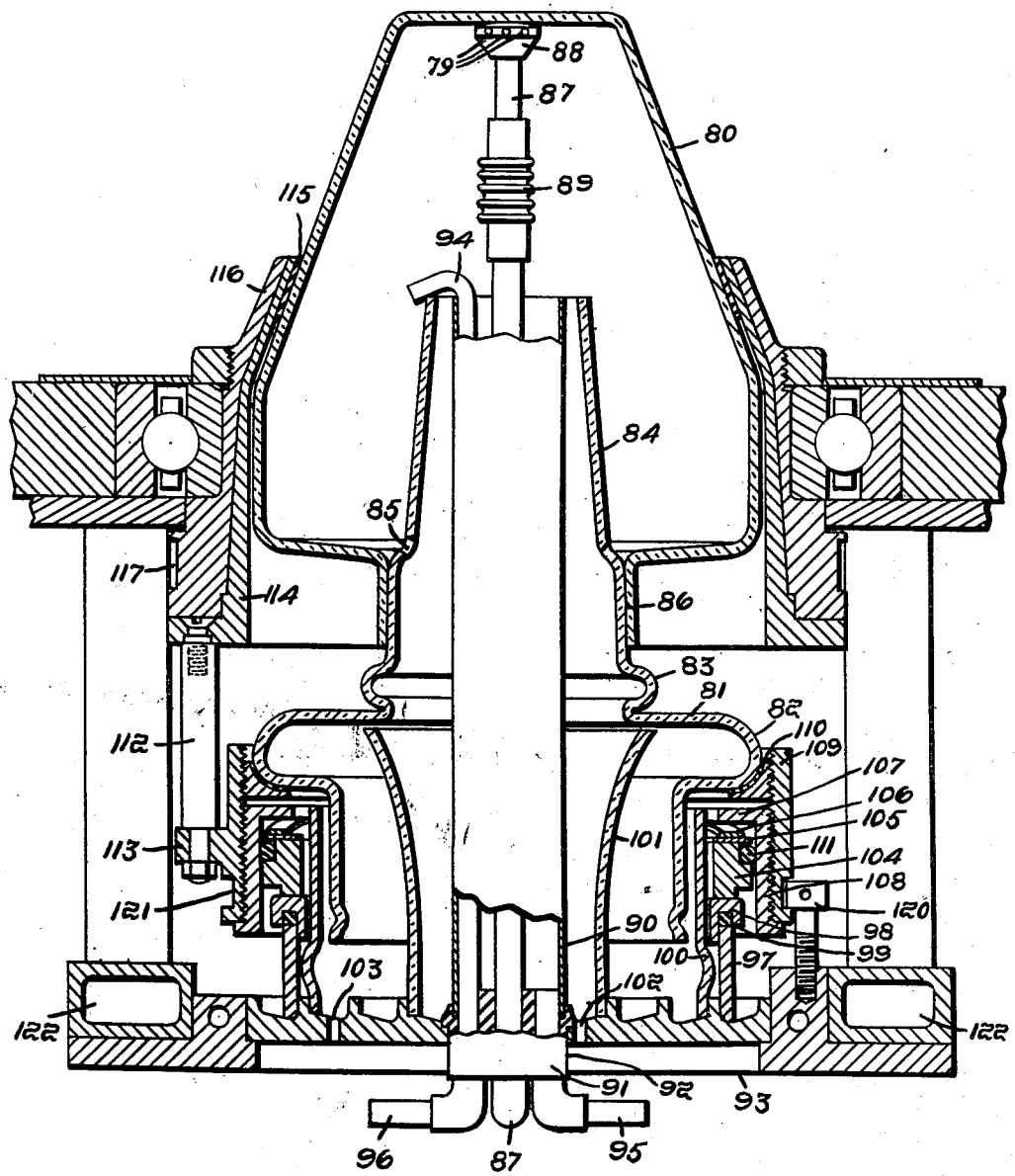
Fig. 3.



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CONTINUOUS FEED CENTRIFUGE

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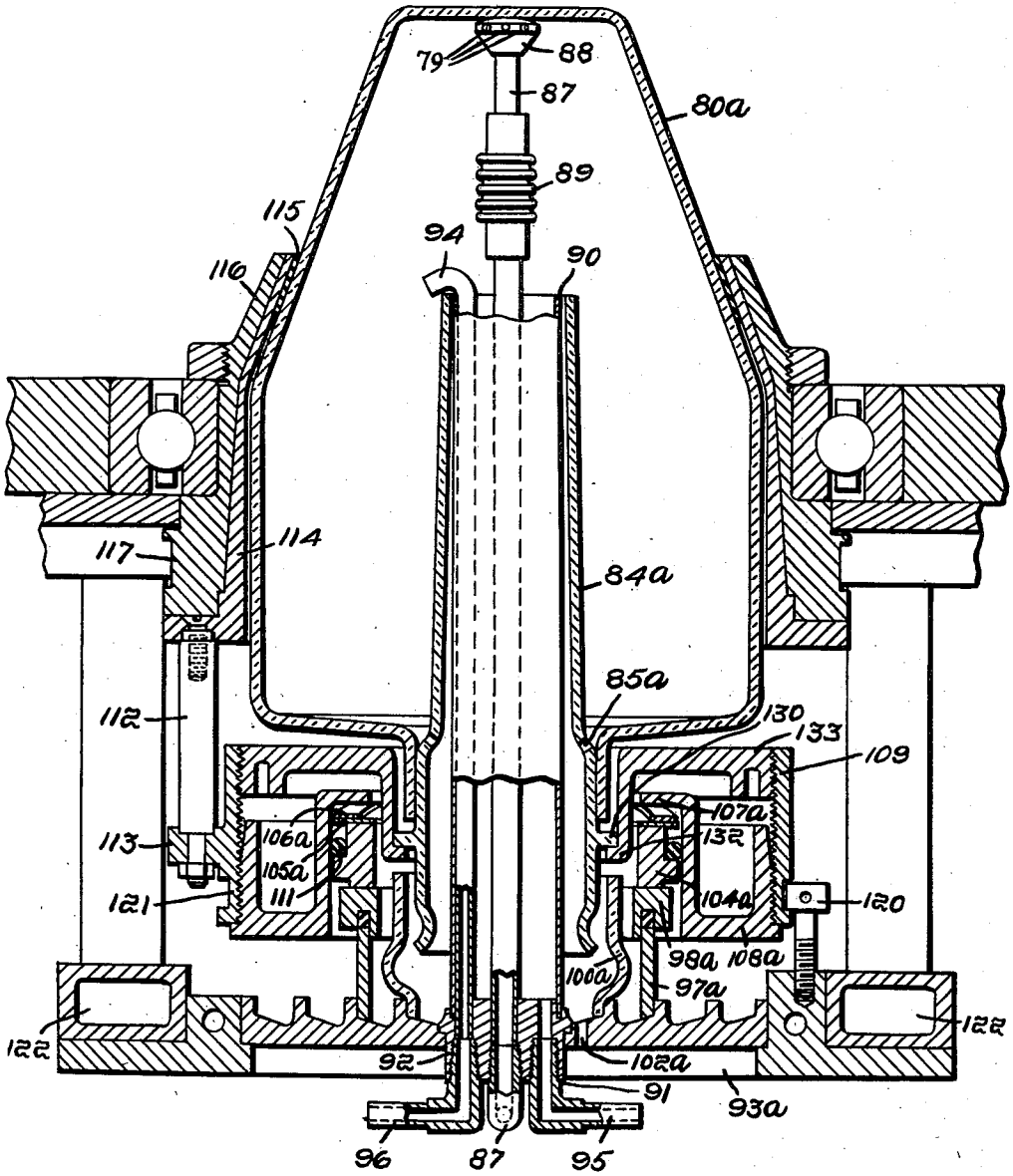
Fig. 4.



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CONTINUOUS FEED CENTRIFUGE

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Application April 12, 1952, Serial No. 281,988

17 Claims. (Cl. 233—1)

This invention relates to centrifugation and, more particularly, to centrifugal apparatus useful in the separation of the various components of animal blood, though it may equally well be used in the separation of components, having different specific gravities, of other liquids; and to methods effecting separations by centrifugation.

Not only is the apparatus of this invention novel, but also the methods of separation carried out in operation of the devices constitute wholly new methods of tremendous value in effecting separation of the cellular components of animal blood from plasma, as well as in the separation of the various protein constituents of plasma.

A requisite for optimum preservation of blood plasma is separation not only of the platelets, but also of the other cells therefrom.

Early methods for separation of the cellular components of the blood from the plasma depended wholly upon sedimentation under the action of gravitational force. Such methods were originally extremely slow but the time factor was somewhat improved by the addition of reagents conducive to rouleaux formation of the red cells.

Extensive experience with blood has indicated, however, the grave significance of rapid techniques when the objective is recovery of the components most nearly in their state in nature, i. e. in the state in which they exist in the body. Only a few moments elapse before the optimal conditions for preservation of the blood components are dissipated. It has also become known that undesirable changes in the nature of the components are minimized by conducting operations at low temperatures. Such low temperatures so increase the viscosity of plasma that sedimentation operations, even with rouleaux formation, have been too slow.

Accordingly, centrifugation was attempted with available types of centrifuges. In one type, bottles, each holding a blood donation of approximately 500 cc., were balanced out about an axis of rotation and the red cells collected near the bottom of the bottles. Speeds of about 2,000 R. P. M. were used and separation was performed in about 20–30 minutes. It was entirely a batch operation and the red blood cells could readily be damaged by too high speeds. Sterility maintenance is achieved, when this type of centrifuge is used by exercising great care in drawing off the separated components from the bottles; generally in a sterile room, or under a sterile hood. This is necessary since the processing cannot be completed in a closed system, and every transfer is attended by the risk of bacterial or virus contamination.

Objects of this invention include the provision of a centrifuge wherein a separation of cellular components of blood may be achieved in a rapid manner even at low temperature, wherein the operation is continuous, and wherein both the plasma and cellular components may be recovered under aseptic conditions in an essentially closed system.

Apparatus of this invention is so versatile that not only may plasma be separated from red cells, but also white cells may be concentrated, separated from both red cells and plasma, and a thorough washing of the red cells may be carried out to free the cells substantially completely of plasma, thus not only increasing the plasma yield, but isolating the red cells from the continued presence of plasma and thereby probably prolonging their viability.

In the accompanying drawings Fig. 1 is a diagrammatical cross sectional representation of the action of a simplified form of centrifuge of this invention;

Fig. 2 is a similar view of the action of a modified form of the invention including provision for separate collection of the white blood cells;

Fig. 3 is a cross sectional view of a commercial embodiment of the device shown in Fig. 2 with a different form of air seal, and;

Fig. 4 is a cross sectional view, also with a modified form of air seal, of a centrifuge designed for particular use in the separation of proteins.

The design of the present centrifuge evolved from experience with previous centrifuges, which established certain requirements preserved in the present centrifuge. First of these requirements stems from the discovery that the viability of the cellular components is greatly enhanced by, if not wholly dependent upon, the utilization throughout the equipment of non-wetting surfaces. Such surfaces may be of Teflon (tetrafluorethylene), but both metal and glass may be satisfactorily coated with silicones, forming highly satisfactory, though not particularly durable, surfaces. The surfacing requires renewal.

In Fig. 1, I have shown a typical basic shape for a centrifuge useful in accordance with this invention. The present device deviates from a cylindrical surface and is cone-shaped with a closed top **20**, the wall **22** being flared outwardly downwardly and the container being provided at its bottom with an outlet **24** of restricted area.

In the processing of a single blood donation, generally 500 cc. of blood, there is a relatively large volume of red cells to be separated, namely, in the neighborhood of 200–225 cc. out of the total 500 cc. of blood. In order to retain such a volume of red cells, it is necessary that a cylindrical centrifugal bowl be of considerable height with the result that towards the end of the processing, the held-up volume of red cells is relatively large. Studies indicated that despite the centrifugal force, gravity had considerable influence upon the held-up volume with the result that the red cells, packed near the bottom of the centrifuge, sustain an undesirable amount of weight and, additionally, there tended to be an inward packing towards the bottom of the centrifuge so that the straight falling-film effect was negatived towards the end of the donation. There was additional tendency towards the end of the donation for the red cells at the bottom to slip over the outlet along with the plasma. First attempts were along the line of reducing the height of the cylinder, but the effects were not wholly acceptable. On the contrary, in the case of a conical surface as here used, a much smaller fraction of the total cells are collected near the top of the centrifuge and, hence, there is less cell weight exerted on those collected near the bottom of the vessel.

Thus, if one feeds calcium-free or calcium-bound blood into this rotating vessel upwardly through a central non-rotating inlet tube **30** so that the blood flows out of the tube **30**, through a flexible end portion **31**, against the top of the centrifuge bowl, a body of blood will build up against the conical side wall **22**, as shown at the left of Fig. 1, until at a later time its inner diameter is re-

duced to the diameter of the bottom outlet **24**, as shown at the right in Fig. 1. Continued introduction of blood will result in the blood flowing down the inner cylindrical surface of this rotating body of fluid and the rate of flow and distance thereof may be such that by the time the liquid reaches the restricted outlet **24** at the bottom, most or all of the cellular components, depending upon the rate of rotation and of flow, will have passed into the volume of the vessel in which fluids or suspensions are retained while centrifuge action continues, displacing any material, mostly plasma, of less specific gravity.

If the size of the container is such that the volume outside of a central cylinder having the diameter of the opening **24**, is exactly equal to the volume of the cellular components of the blood, a substantially complete separation of the cells will theoretically take place.

However, substantially complete separation of plasma is not achievable even if the force of centrifguation and the time be as great as in the cup or cylindrical falling-film type of centrifuge, for there is always occlusion of plasma between even the most tightly packed cells or particles, and tight packing is injurious to cells and renders difficult resolution of precipitates.

Experience has shown that the theoretical separation is impossible to achieve and, hence, a compromise is made in determining how tightly packed the cells or particles should be. This is determined in the new blood separators by the retaining volume of the centrifuge vessel; the flow rate and the centrifugal force determining the time necessary to achieve the desired separation. Thus, if the cell volume of the 500 cc. blood donation be 225 cc. and the retaining volume of the separator bowl be 325 cc. the hematocrit is just under 70 and the plasma yield only 64 percent; if the retaining volume is 300 cc. the resulting hematocrit is 75 and the plasma yield 73 percent; if the retaining volume is 275 cc. the hematocrit is nearly 82 and the plasma yield is also 82 percent.

In the past, when a high plasma yield was desired, high centrifugal forces have been used for long times, probably to the detriment of labile cellular elements; when the cells were most desired a lower hematocrit has generally been chosen, with the result that the yield of plasma was uneconomically low. Centrifugal forces utilized, in accordance with this invention, are of the order of 40 to 200 × gravity for red and white cell separations.

Any of these separations are readily achieved in the new centrifuges at the usual flow rate of 50 cc. a minute, though more rapid or slower flow rates may be achieved with equal ease, should this be desirable for any separation. The independence of hematocrit and the time of centrifugation is a great convenience. It should be pointed out, however, that the lower the centrifugal force, the greater the angle of the separator bowl should be in order that the plasma, at the end of the centrifugation not be trapped by the very dense red cells sedimenting under the downward force of gravity and obstructing the falling-film path of the plasma—thus diminishing plasma yield—and red cells even passing into the collecting cup; thus rendering unachievable the goal of a quantitative yield of cell-free plasma and without injury to the formed elements of the blood.

The choice of optimal angle in the bowl is important for the lowest centrifugal force that will permit quantitative separation of cells or particles of any specific gravity and shape in a vessel of the desired retaining volume without such vibrations in the centrifuge which might cause red cells to slip over with the plasma near the end of the collection.

In Fig. 2, a still further improved type of device is shown intended for use in separating not only plasma from red blood cells, but also for concentrating white blood cells separated from red blood cells. The upper portion of the centrifuge remains the same, but there is added a second flared portion **40**, in addition to the inner cylindrical retarder and separator tube **42**. The shape and volume of the second vessel can be varied, as can the first bowl, with the specific gravity of the cells or particles to be collected, with their concentration in the solution, in which they are suspended, in order to separate them most effectively from other cells or particles in the fluid and concentrate them to the desired volume. Even after determining the volume in which they are to be concentrated, the choice of shape of each succeeding collecting bowl can be varied in order to give the cell or particle adequate time, at the centrifugal force chosen, to traverse the separator and be concentrated at, or near, the outer wall of the rotating bowl. Indeed, multiple bowls may differ in diameter, volume and length to accomplish the separation of the many cells of various tissues.

The following centrifugal forces have been employed in separating the various formed elements of the blood from the plasma. The three most important types are:

Formed Elements	Percent of Volume in Normal Blood	Specific Gravity	Centrifugal, Force to Separate them from Plasma
Red blood cells	45	1.090	60
White blood cells	1	1.045	200
Platelets	0.1		600
Plasma	54	1.026	

In order to render such separations even more effective the rotating upper bowl, before the beginning of the operation is charged with a priming solution which has a higher specific gravity than the plasma, though lower than that of the red blood cells. The specific gravity of the white blood cells is intermediate between red cells and plasma and the extent to which they penetrate the high density layer depends upon its specific gravity. Hence, when blood is introduced, only the red blood cells will rapidly pass into and traverse the high density solution as shown at the left in Fig. 2. As the feed continues, the condition shown at the right in Fig. 2 will prevail and both the plasma and white blood cells will pass down the inner rotating interface of the high density solution, as a falling-film, and through a series of small apertures **44** in retarder tube **42** into the outlet **24** and back out through a series of apertures **46**. The separation between white blood cells and plasma takes place in the lower bowl **40**, the diameter of which gives a centrifugal force adequate to retain the white blood cells. In the assembly in Fig. 2, the white blood cells would have been retained at the outer edge of the upper bowl if they had not been restrained from reaching it by the high density solution and by the more dense red blood cells. The plasma flows uninterruptedly through outlet **50**. After the plasma has been withdrawn during the centrifugation, the bowls are stopped and the white blood cells will then pass out the same outlet **50** as the plasma, and the red blood cells will flow directly downwardly to outlet **52**, still contained in the high density solution. While the centrifuge is being filled up, separation occurs in a location that moves gradually upwards and inwards.

Since centrifugal force decreases as the radial distance, movement across the film will be slower the fuller the bowl, as at the end of the operation. The inner edge of the retaining volume is, therefore, chosen to have a force adequate for the separation of the red cells, which at the very end penetrate the high density solution from the falling film, the distance to the outlet then being maximum.

The presence of the high density solution offers the advantage of providing an excellent washing for the red blood cells. This solution can be so constituted that it contains in addition those ingredients most desirable for subsequently preserving the red cells (globin, serum

albumin, or other protein colloid); and the purposes of better separation, it has at times contained rouleaux-forming agents. Among the rouleaux-forming agents that are satisfactory is an amylose or dextrin and glut-amylpeptide. Useful priming solutions are 14% lactose, 4% amylopectin solution or a 14% lactose, 0.28% sodium chloride solution. Electrolytes such as potassium and bivalent cations that are necessary for the preservation of the red cells, and that have been removed during the passage of blood over the cation exchange resin may also be reintroduced in the high density solution into which these cells enter within a second after leaving the resin. The ease of variation in the components and their quick introduction into the red blood cells is of great importance.

In the case of the centrifuge of Fig. 2, for a 500 cc. donation the capacity is desirably 325 cc., the high density solution used during collection having a volume of about 100 cc.

Figs. 1 and 2 also illustrate further features of the centrifuge including a series of concentric baffles **60** to aid in excluding contaminants from the air, the exterior baffles, if desired, containing to the level shown a liquid seal such as a body of silicone oil **62**, either for aiding against contamination or for maintaining internal pressure.

There is also provided a gaseous inlet comprising a central tube **70** having an outlet **72** near the top of the bowl for charging the system with nitrogen, oxygen or other gaseous medium at any desired pressure for any desired purpose.

Centrifuges built on this same principle are also used in performing fractionation of the separated plasma after the addition of chemical reagents.

These centrifuges may, however, be of the simpler Fig. 1 type because the proteins are separated in smaller quantity and, hence, slipping effects due to the action of gravity are almost negligible; and, anyway, much higher centrifugal forces may be used than in the case of the tender cells, so that the ratio of centrifugal force to gravity may be much higher.

The centrifugal bowl of Figs. 1 and 2 may be mounted in a suitable enclosed and refrigerated housing; the upper rotating bowl portion may be suspended through a rubber-lined ball-bearing supported annulus and driven by a belt and pulley arrangement from a motor or by a friction drive, the lower collecting portion being mounted on a suitable stationary frame. The mountings are omitted in Figs. 1 and 2, since mountings are shown in Figs. 3 and 4.

In Fig. 3, the same centrifuge principles are incorporated, but the device of Fig. 3 includes a different form of seal more convenient from a handling standpoint. The assembly is also designed so that its operating parts may more easily be sterilized.

As in the case of Fig. 1 and Fig. 2, the essential parts include a closed-top, conical side wall, internally silicone-coated glass container **80** having a circular restricted bottom opening.

A second tubular member **81** of silicone-coated glass is inserted upwardly in the bottom opening, the part **81** having a lower section of enlarged diameter **82** for the collection of white blood cells and also having another section of intermediate diameter **83** which acts as a catch for any red cells which may slip from the upper bowl, particularly towards the end of a donation.

The upper portion **84** acts in the same manner as the element **42** in Fig. 2 as a retarder and has in it a series of circumferentially spaced apertures **85** corresponding to the apertures **44** in the device of Fig. 2.

The parts **80** and **82** are provided with ground interfitting surfaces at **86**. The central stationary feed tube **87** has at its top a convex-surfaced cap **88** having outlet apertures **79** below the top of its convexity so that the tube **87** may lightly touch the top wall of the bowl **80** without interfering with the flow of blood into the bowl. A bellows-type connector **89** interposed in the tube **87** provides longitudinal yieldability. The tube **87**, together with a surrounding sleeve **90**, are both seated in a fixture **91** which fits downwardly into a bevelled aperture **92** in a bottom plate **93**. The fixture **91** also supports a second tube **94** so that the combined fixture provides three separate inlets to the bowl chamber, the tube **87** being for blood, the sleeve **90** being connected to an inlet **95** for gas inflow and the tube **94** being connected to a tube **96** for introduction of reagents, if required.

The stationary plate **93** also carries fixed thereto an upstanding cylindrical glass support **97** on top of which is seated a stainless steel annulus **98** with an interposed soft gasket **99**.

The base plate also supports a concentric baffle **100** and upwardly flared separator **101**. Outlets through the base plate are provided at **102** and at **103**, one inside and one outside the separator **101**.

A sealing surface with annulus **98** is provided at the bottom lapped surface of a compressed graphite ring **104** which bears upwardly against a bearing ring **105** against the yield of an undulating annular metallic spring **106** seated against flange **107** of an internal housing **108**. A gasket **111** is interposed between ring **104** and housing **108**. The housing **108** is screw-threaded into an exterior sleeve **109** which has connected thereto a curved annular rubber gasket **110** for supporting the lower portion of the glass element **82**.

All the elements **104—110** rotate with the upper bowl by virtue of three circumferentially spaced connecting pins, one of which is shown at the left in Fig. 3 and at **112**, being threaded through a boss **113** on the housing **109**, thus attaching the lower portion of the assembly **104—110** for rotation with an inside upper sleeve **114** which engages the upper bowl **80** through an interposed rubber gasket **115**. An outside pulley sleeve **116** is designed to receive the inside sleeve **114** with frictional engagement, thus supporting the bowl **80** and its assembled parts for rotation. The sleeve **116** is supported in ball bearings and driven by a belt (not shown) engaging the pulley groove **117**.

In order to hold the upper and lower parts of the assembly which can rotate relative to one another, against vertical separation, bolts **120**, one of which is shown at the right in Fig. 3, are provided having lost motion connection with a groove **121** in the external sleeve **109**.

As shown, parts of the stationary frame may be provided with internal conduits **122** for the circulation of coolants.

The collector plate **93** may be clamped up against the stationary frame in any conventional manner.

Fig. 4 shows a centrifuge wherein many of the parts are duplicates of the centrifuge shown in Fig. 3, the devices being built so that these parts are interchangeable. However, the dimensions of the bowl are different and, accordingly, the collector plate and the seals are differently proportioned. The stationary central tube parts have the same three infeeds, including the parts **87** through **96**, identical with the same parts shown in Fig. 3. The same is true of the parts **109**, **112—117**, **120** and **121**. The bowl **80*a*** is, however, of much greater vertical dimension and has a slightly smaller bottom opening and, therefore, it has a longer retarder **84*a***. In this case, the lower part of the member **84*a*** has an outwardly extending flange **130** which engages a lip **132** on the sleeve **133** which is screw-threaded to the external housing **109**. The member **108*a*** corresponding to the internal sleeve **108** in Fig. 3 is differently dimensioned, as are the smaller undulating spring **106*a***, sealing ring **105*a*** and compressed graphite ring **104*a***, but, otherwise, these parts, as well as the collector plate parts **97*a*** and **98*a***, function in the same manner as the corresponding parts in Fig. 3.

In this case, there is need of only one opening **102***a* in the base plate **93***a* inasmuch as the centrifuge is designed principally for the separation of proteins and in which a precipitate is left in the bowl **80***a* and soluble components pass downwardly to the apertures **85***a* and outlet **102***a* continuously after the bowl has been filled so that its internal rotating wall surface is at the radius of the apertures **85***a*.

I claim:

1. A centrifuge comprising a closed-top vessel having a bottom wall, a central opening in the bottom wall forming an outlet of restricted area, and side walls connected to said bottom wall and having conical portions converging towards the closed top of the vessel, stationary means extending upwardly through said opening to a point near the top of said vessel for feeding liquid into said vessel and discharging the liquid adjacent the central portion of the top wall thereof and means disposed below said vessel for collecting liquid discharged from said vessel through the restricted bottom outlet thereof, said vessel being adapted for rotation about a vertical axis.

2. A centrifuge as claimed in claim 1, wherein said collecting means is stationary and said vessel and said collecting means have an interposed seal against ingress of air.

3. A centrifuge as claimed in claim 2, wherein said seal comprises an annular body of liquid material carried between interlocking vertically extending baffles on said vessel and collecting means.

4. A centrifuge as claimed in claim 1, wherein a cylindrical section of said vessel of restricted diameter extends downwardly from said bottom wall and defines said outlet, said centrifuge also including radially outwardly extending walls below said cylindrical section forming an additional rotatable section of increased diameter below said cylindrical section, and a tubular separator of substantially the same diameter as said cylindrical section disposed below but vertically spaced from said cylindrical section.

5. A centrifuge as claimed in claim 4, wherein said collecting means is stationary and is coaxially aligned with said vessel and has a series of outlets, one of said outlets being located at a radius less than the radius of said separator and the other of said outlets being at a radius greater than that of said separator.

6. A centrifuge comprising a closed-top vessel having a bottom wall, a central opening in the bottom wall forming a circular outlet of restricted area, and side walls connected to said bottom wall and having conical portions converging towards the closed top of the vessel, a vertical retarder tube extending upwardly from said circular outlet and having a series of apertures disposed circumferentially thereof adjacent said circular opening, stationary means extending upwardly through said outlet and retarder tube to a point near the top of said vessel for feeding liquid into said vessel and discharging the liquid adjacent the central portion of the top wall thereof and means disposed below said vessel for collecting liquid discharged from said vessel through said apertures and the restricted bottom outlet thereof, said vessel and retarder tube being adapted for rotation about a vertical axis.

7. A centrifuge as claimed in claim 6, wherein said collecting means is stationary, and sealing means interposed between said rotating vessel and said stationary collecting means for maintaining the same aseptically sealed during relative rotation thereof.

8. The method of continuously separating plasma from the cellular components of blood comprising flowing non-clotting blood upwardly into the top portion of an inverted closed-top rotating bowl having a central bottom outlet of restricted area and discontinuing the feed before the volume of cells packing on the side walls of the rotating bowl as the blood flows downwardly attains an internal diameter as small as the diameter of said bottom outlet.

9. The method of continuously separating plasma from the cellular components of blood comprising flowing non-clotting blood into a rotating bowl having a central bottom outlet of restricted area, said bowl containing a priming solution held up by the centrifugal force imparted by rotation of the bowl, said priming solution being of greater density than the plasma of the blood but of less density than that of the red cell components of the blood, and collecting plasma as it flows from said bottom outlet.

10. The method of continuously separating plasma from the cellular components of blood comprising flowing non-clotting blood upwardly into the top portion of an inverted closed-top rotating bowl having a central bottom outlet of restricted area, said bowl containing a priming solution held up by the centrifugal force imparted by rotation of the bowl, said priming solution being of greater density than the plasma of the blood but of less density than the red cell components of the blood, and collecting plasma, as it flows from said bottom outlet.

11. The method as claimed in claim 10, wherein the priming solution contains rouleaux-forming agents.

12. The method of treating a liquid for the separation therefrom of substances of different specific gravity comprising forming an annular body of liquid rotating on a vertical axis and having a substantially cylindrical inner wall and a substantially triangular cross-section, the base of said triangle extending outwardly from the bottom of said inner wall, at least some of the components of said rotating body having a specific gravity less than that of at least some of the components of said liquid to be treated, and continuously feeding the liquid to be treated outwardly against the top of said inner wall and flowing it in the form of a film down and off of said inner wall to displace material of less specific gravity contained in said rotating body with material of greater specific gravity contained in the liquid flowing down said inner wall, collecting the effluent as it flows from the bottom of said inner wall, and discontinuing said feed before the volume of material separated from the liquid being treated exceeds the volume of the rotating annular body.

13. The method of separating substances of different specific gravity contained in a liquid comprising continuously flowing said liquid down the internal cylindrical wall of an annular body of liquid rotating on a vertical axis and having a substantially triangular cross-section, the base of said triangle extending outwardly from the bottom of said internal wall, the liquid in said rotating body having a density intermediate those of the substances to be separated and gradually displacing the material of intermediate density with substances of greater specific gravity contained in said continuously flowing liquid while collecting substances of less specific gravity as they flow from the bottom of said internal wall.

14. A centrifuge comprising a closed top vessel having an annular bottom wall with its central opening providing an outlet for said vessel, stationary means extending upwardly through said opening for feeding liquid into said vessel, and means disposed below said outlet for collecting liquid discharged from said outlet at a radius greater than that of said outlet while the centrifuge is rotating and within the radius of said outlet when said vessel is stopped, said vessel being adapted for rotation about a vertical axis, whereby liquids discharged through said outlet during spinning of said vessel may be collected separately from liquids discharged therethrough when said vessel is stopped.

15. The method of continuously removing red cells from blood comprising flowing non-clotting blood upwardly into the top portion of an inverted closed-top rotating bowl having a central bottom outlet of restricted area to form a hollow body held up in the bowl by centrifugal force imparted by rotation of the bowl, con-

tinuing feeding the blood after the body attains an internal diameter equal to that of said bottom outlet so that the further blood fed flows continuously downwardly across the inner face of the rotating body and centrifuging the downwardly flowing blood to divert red cells outwardly away from the plasma into the body and collecting undiverted red cell-free plasma as it flows continuously from said bottom outlet and discontinuing the feed of blood before the volume of red cells packing on the side walls of the rotating bowl attains an internal diameter as small as the maximum diameter of said bottom outlet.

16. The method as claimed in claim 15 wherein the blood is at a temperature of from 0–5° C.

17. The method as claimed in claim 15 wherein the bowl has a frusto-conical configuration to suspend the blood in the form of a hollow body of generally triangular vertical cross-section.

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