United States Patent Office

2,833,691

Patented May 6, 1958

2,833,691

BLOOD PRESERVATION BY ION EXCHANGE RESIN DECALCIFICATION

Nicholas Paul Klaas, Pennsauken, N. J., and Arnold E. Osterberg, Waukegan, Ill.; said Klaas assignor to Rohm & Haas Company, Philadelphia, Pa., a corporation of Delaware, and said Osterberg assignor to Abbott Laboratories, North Chicago, Ill., a corporation of Illinois

No Drawing. Application July 24, 1951
Serial No. 238,404
5 Claims. (Cl. 167—78)

This invention relates to the preservation of freshly drawn whole blood, and more particularly to the decalcification and maintenance of sodium-potassium ionic balance of whole blood by means of an ion exchange resin.

Whole blood, whether fresh or stored, is generally more useful in replacing the loss of circulating blood volume than any of the known blood fractions or blood substitutes. The use of blood preserved and stored at refrigerating temperatures has become the salient feature of transfusion therapy. Stored blood is generally useful for about twenty-one days, making it possible to keep an adequate supply available for emergencies and general uses.

One of the necessary prerequisites to the storage of whole blood is the prevention of coagulation of the blood. Of the several methods of preventing coagulation, the employment of a chemical anticoagulant has been most widely used. Such anticoagulants include tri- and di-sodium citrate, heparin, etc. The use of such chemical anticoagulants, however, tends toward an unbalance of the normal blood ingredients.

It is the general practice to add the blood to an aqueous solution of an anticoagulant in order to maintain the stability of the blood by preventing clotting, etc. Furthermore, blood is normally diluted with nonelectrolytic solutions to inhibit spontaneous hemolysis. Dextrose has been found to be an effective nonelectrolytic diluent, as it not only inhibits hemolysis but serves as a substrate for the glycolytic enzymes of the erythrocytes. The dextrose, furthermore, appears to provide energy for the metabolism of the cells during the storage period.

The use of citrated blood, i. e. blood preserved with sodium citrate, may result in over-citration and upset of the electrolytic balance of the blood, so that potassium, sodium, etc. diffuse through the cell membrane increasing cell rupture or hemolysis. In large volume blood transfusion, the use of citrated blood is contraindicated, due to upsetting the electrolytic balance and the possibility of hemolysis in the body.

Included among the objects and advantages of this invention is a novel and efficient method of decalcifying whole blood to prevent coagulation thereof, and a method of maintaining the balance of potassium to sodium ions in the plasma so as to prevent increased cell fragility and excessive hemolysis during storage of the whole blood.

According to the present invention, whole blood, immediately on withdrawal from the donor, is passed through a cation-exchange resin which is predominantly in the form of the sodium salt, to a much less extent in the form of the potassium salt. The resin substantially removes the calcium ions from the blood plasma, thereby preventing coagulation of the blood and subsequent deterioration. The small amount of the resin, which is in the form of the potassium salt, maintains the normal potassium-sodium ratio of the blood plasma and the blood cells. The decalcified blood is then run into suitable storage containers, containing a small amount of dextrose, and kept under refrigeration.

In the storage of whole blood it is imperative that the

freshly drawn blood be treated with an anticoagulant immediately on withdrawal from the donor. Normal blood spontaneously starts the clotting procedure immediately on leaving the body, especially when contacted with steel, rubber, glass and the like. It is, therefore, desirable to use the ion exchange resin column connected directly in the bleeding tube used to withdraw blood from the donor. Such a resin column should be as close as possible to the vein of the patient.

The following examples will illustrate the invention in more detail:

Example I

A small glass or synthetic plastic ion exchange resin column, of about 20 cc. capacity, is connected in a length of tubing having a hypodermic needle attached to each end thereof. The column is about 0.620 inch diameter by 3.5 inches in length, and is filled with about 15 cc. of a cation-exchange resin. The cation-exchange resin which was employed was one in which the polar groups were carboxyl groups, and which was made by copolymerizing about 95% methacrylic acid and about 5% divinylbenzene. About 96% of the resin is in the sodium salt form, while 4% by volume is in the potassium salt form, and it is buffered to pH 7.2–7.4. The resin is prepared by blending the desired proportions of the potassium salt form with the sodium salt form. The resin is separately converted to the sodium and potassium form by washing a portion with an aqueous solution of sodium hydroxide, carbonate or bicarbonate, and the remainder converted to the potassium form by washing with potassium hydroxide, carbonate or bicarbonate. The blended potassium and sodium salt form resins are buffered by washing to a constant pH of 7.2–7.4 with a standard citric acid-phosphate buffer solution.

After being sterilized, the outlet end of the tube is connected by means of the hypodermic needle to a 550 cc. bottle, containing 1.84 gms. of dextrose. The needle on the inlet end is then inserted in the vein of the donor, and blood flows from the donor, through the column of resin and into the bottle.

Within very narrow limits, human blood contains about 5.0 milli-equivalents per liter (m. eq./liter) of potassium ion in the plasma fraction, and about 4.79 milli-equivalents of calcium ion per liter. The following table shows the analysis of blood, collected through an ion exchange column as above, from a group of typical donors. In each case the blood was stored at about 5° C. for twenty-one days, and there was no sign of clotting in any of the samples. There was little or no hemolysis in the samples, showing good storage qualities of the decalcified blood. In all cases the calcium removal was better than 99%.

Donor	Plasma Potassium, Milli-equivalents per liter	Plasma Calcium, Milli-equivalents per liter
1	5.45	<0.05
2	7.00	<0.05
3	6.58	<0.05
4	5.28	<0.05
5	7.25	<0.05
6	6.75	<0.05
7	6.8	<0.05
8	6.25	<0.05
9	6.75	<0.05
10	7.6	<0.05

Example II

About 500 cc. of blood was collected from a donor through a 15 cc. ion exchange column (similar to Example I) containing a carboxylic cation-exchange resin which was entirely in the sodium salt form. Analysis of the plasma of collected blood showed greater than 99% removal of calcium, but the potassium content of the

plasma had dropped from the normal 5.0 milli-equivalents per liter to 0.57 milli-equivalent per liter. On storage, hemolysis was apparent in a few hours, and there was strong hemolysis in about 10 days showing that the blood cells were friable. The extent of the hemolysis made the blood practically valueless for transfusions.

Example III

Following the procedure of Example I, about 500 cc. of blood was treated with about 25 cc. of the same kind of carboxylic cation-exchange resin. Two percent of the resin by volume was in the form of the potassium salt and 98% was in the form of the sodium salt, and the resin was buffered to pH 7.2–7.4. The following table shows the analysis of the blood after treatment.

Donor	Plasma Potassium, Milli-equivalents per liter	Plasma Calcium, Milli-equivalents per liter
11	4.74	<0.05

The amount of resin which will remove substantially all the calcium from the standard donor quantity (500 cc.) of blood may be as little as 10 cc. For instance, 10 cc. of resin containing about 3% of the potassium salt, the remainder being in the sodium form, removes calcium from 500 cc. of blood to approximately the same degree as the 25 cc. resin column of Example I, i. e. greater than 99%, and also maintains the potassium content of the decalcified blood at an average of 5.2 m. eq./liter. However, it is preferable to use at least 15 cc. of resin per 500 cc. of blood to insure sufficient adsorption capacity for the calcium, due to the value of human blood.

The invention has been found to be operable where the amount of the potassium salt form is 2–10% by volume, the remainder being in the sodium salt form. The optimum amount of the potassium salt form has been found to be 2–5% by volume, as in this range the normal potassium-sodium ion ratio of the plasma is substantially unchanged by the resin treatment of the blood. In certain types of diseases, however, increased potassium may be indicated, in which event the amount of the potassium salt form of the resin may be increased. For instance, by using about 10% by volume of the potassium salt form of the resin, the remainder in the sodium salt form, the plasma potassium content is increased from about 5.0 m. eq./liter to about 12.5 m. eq./liter.

The cation-exchange resin which is used in the decalcification of blood by the process of this invention may be either of the sulfonic type or the carboxylic type; that is, a resin in which the polar groups are either sulfonic groups or carboxyl groups. We prefer, however, to use the carboxylic type cation-exchanger because of its greater affinity for calcium ions in the presence of other metal ions. The carboxylic type cation-exchange resins that are particularly suitable for use in this fashion are the cross-linked polymers of polymerizable acids, particularly the cross-linked polymers of acrylic acid, methacrylic acid, and maleic acid. These exchangers can be made by the joint polymerization of a polymerizable acid and a divinyl aromatic compound by the method described in U. S. Patents Nos. 2,340,110 and 2,340,111. Other carboxylic exchangers are formed by the condensation of hydroxy benzoic acids with formaldehyde. We prefer to employ a resin formed by the suspension copolymerization of from 90–97.5 parts of acrylic or methacrylic acid with from 2.5–10 parts of divinylbenzene. The sulfonic type cation-exchangers include carbonaceous zeolites and sulfonated synthetic resins. The resins are for the most part sulfonated phenolaldehyde products such as are described and disclosed in U. S. Patents Nos. 2,184,943; 2,195,196; 2,204,539; 2,228,159; 2,228,160; 2,230,641; 2,259,455; 2,285,750; 2,319,359; and 2,361,754.

Also included are the sulfonated, cross-linked polymers of styrene which are made according to the process of U. S. Patent No. 2,366,007. All of these resins are characterized by being insoluble and infusible and capable of sorbing cations from fluids.

Others may readily adapt the invention for use under various conditions of service, by employing one or more of the novel features disclosed or equivalents thereof. As at present advised with respect to the apparent scope of our invention, we desire to claim the following subject matter.

We claim:

1. In the method of preserving whole blood, the step which essentially comprises, contacting blood, immediately on withdrawal from a living body, with a mixture of cation exchange resins buffered to pH 7.2–7.4, said mixture comprising about 2%–10% on a volume/volume basis of the potassium salt form thereof, and the remainder of the mixture in the sodium salt form thereof, whereby the calcium ion of the blood plasma is substantially absorbed on the cation exchange resin, and the blood plasma and blood cell potassium-sodium ionic balance is substantially maintained.

2. In the method of preserving whole human blood, the step which comprises, removing substantially all the ionic plasma calcium from blood, by contacting said blood immediately on withdrawal from a human body with a mixture of cation exchange resins, said mixture consisting essentially of 95%–98% on a volume/volume basis of the sodium salt form of a carboxylic cation exchange resin and 2–5% on a volume/volume basis of the potassium salt form of the resin, whereby the potassium-sodium ionic balance of the decalcified blood is substantially the same as the original whole blood.

3. In the method of preserving whole human blood, the step which comprises, contacting whole human blood, immediately on withdrawal from a living body, with a mixture of cation exchange resins buffered to pH 7.2–7.4, whereby ionic plasma calcium is substantially removed from said blood, said resin being present in an amount of at least 10 cc. per 500 cc. of blood so withdrawn, said mixture comprising 2–10% of resin on a volume/volume basic in the potassium salt form and the remainder in the remainder in the sodium salt form, whereby the potassium-sodium ionic balance of the decalcified blood is substantially the same as the original whole blood.

4. In the method of preserving whole blood, the step which comprises contacting blood, immediately on withdrawal from a living body, with a mixture of cation exchange resins, said mixture containing 90%–98% on a volume/volume basis of a cation exchange resin in the sodium form and 2%–10% on a volume/volume basis of a cation exchange resin in potassium form.

5. In the method of preserving whole blood, the step which comprises contacting blood, immediately on withdrawal from a living body, with a mixture of cation exchange resins deriving their exchange capacity essentially from carboxylic groups, said mixture comprising 90%–98% on a volume/volume basis of a cation exchange resin in the sodium form and 2%–10% on a volume/volume basis of a cation exchange resin in the potassium form.

References Cited in the file of this patent

Rohm & Haas Co.: Amberlite IRC–50 (1949).

Wintrobe: Clin. Hematology, 2nd ed., 1949, p. 310.

Steinberg: Proc. Exp. Biol. Med., June 1944, pp. 124–127.

Kunin: Ind. & Eng. Chem. 42, January 1950, pp. 68–69.

Ch'en et al.: J. Lab. and Clin. Med., January 1950, pp. 99–110, p. 108 relied on.

Tullis: "Blood Cells and Plasma Proteins" pub. 1953 by Acad. Press Inc., N. Y. C., pp. 207–208, 215–222.